Author:
Burchell, Amy

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Renal Denervation for Resistant Hypertension

Predictors of Procedural Response and Efficacy

Renal Denervation for Resistant Hypertension:
Predictors of Procedural Response and Efficacy

Dr Amy Elizabeth Burchell

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Abstract

Background: Renal denervation (RDN) is an endovascular ablation technique for the treatment of resistant hypertension through disruption of the afferent and efferent renal nerves, thereby abolishing the feedback loop which drives increased sympathetic nerve activity and hypertension. This study aimed to develop measures of technical efficacy for RDN and to identify parameters to guide patient selection for this invasive therapy.

Methods: Autonomic profiling, including measurement of office and ambulatory BP, muscle sympathetic nerve activity (MSNA), heart rate variability, sympathovascular transduction, baroreflex sensitivity (BRS), chemoreflex sensitivity, and markers of inflammation, was carried out at 0,1,3,6 and 12 months, with quantification of aortic distensibility, left ventricular mass and function and cerebral blood flow at baseline and 6 months post-RDN. Procedural success was assessed through abolition of the reflex systemic BP response to intra-renal adenosine infusion (afferent nerves) and of the reflex reduction in renal blood flow in response to a handgrip stressor (efferent nerves).

Results: 18 participants (office BP 192 ± 21/105 ± 23 mmHg, 5.2 ± 1.8 antihypertensive medications) underwent RDN. Office SBP (oSBP) reduced by 16 ± 9mmHg (n=18, p=0.10) and 26 ± 8 mmHg (n=17, p=0.005) at 6 and 12 months post-RDN, respectively. MSNA incidence did not change following RDN (n=11, 61 ± 7 bursts/100heartbeats versus 66 ± 5 bursts/100heartbeats, p=0.47). Baseline oSBP (n=18, R=-0.61, p=0.01) and spontaneous sympathetic BRS (n=13, R=0.56, p=0.045) correlated with the change in oSBP post-RDN. Post-RDN non-responders had an increase in renal vascular resistance not seen in responders (n=7 arteries; 17 ± 5%, p=0.01, n=6 arteries; -9 ± 12%, p=0.49, respectively).

Conclusions: These preliminary studies suggest that patients with a higher office SBP and greater spontaneous sympathetic BRS may respond to RDN, and that an inability to increase renal vascular resistance with handgrip stress after denervation is indicative of disruption of the renal sympathetic nerves.

Dedication and Acknowledgements

This dissertation is dedicated to my family, Chris Foxton, Barbara Burchell, Michael Burchell, Christine Foxton and Robert Foxton, without whose help I would never have been able to complete this project, and to Theo and Jonty who arrived along the way.

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Author's declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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Publications

Dr Amy Burchell was the primary author writing the text for the following manuscripts which were accepted for publication during the course this project, elements of which are comprised in this thesis and referenced accordingly:

- **Burchell AE***,* Rodrigues JCL, Charalambos M, Ratcliffe LEK, Hart EC, Paton JFR, Baumbach A, Manghat NE, Nightingale AK. Comprehensive First-Line Magnetic Resonance Imaging in Hypertension: Experience from a single-center tertiary referral clinic. **J Clin Hypertens**. 2017 Jan;19(1):13-22.
- **Burchell AE**, Chan K, Ratcliffe LE, Hart EC, Saxena M, Collier DJ, Jain AK, Mathur A, Knight CJ, Caulfield MJ, Paton JF, Nightingale AK, Lobo MD, Baumbach A. Controversies Surrounding Renal Denervation: Lessons learned from real-world experience in two United Kingdom centers. **J Clin Hypertens.** 2016 Jun;18(6):585-92.
- **Burchell AE,** Lobo MD, Sulke N, Sobotka PA, Paton JF. Arteriovenous anastomosis: is this the way to control hypertension? **Hypertension.** 2014 Jul;64(1):6-12.
- **Burchell AE,** Sobotka PA, Hart EC, Nightingale AK, Dunlap ME. Chemohypersensitivity and Autonomic Modulation of Venous Capacitance in the Pathophysiology of Acute Decompensated Heart Failure. **Curr Heart Fail Rep.** 2013 Jun;10(2):139-46.

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Table of contents

List of Figures

List of Tables

List of abbreviations

ABPM	Ambulatory blood pressure monitoring
ACE(i)	Angiotensin converting enzyme (inhibitor)
ACR	Albumin:creatinine ratio
ADH	Anti-diuretic hormone
AE	Adverse event
AF	Atrial fibrillation
AHI	Apnoea-hypopnoea index
AngII	Angiotensin II
ANP	Atrial natriuretic peptide
APV	Average peak velocity
ARB	Angiotensin receptor blockers
AT1R	Angiotesin II type 1 receptor
BAT	Baroreflex activation therapy
BBF	Brain blood flow
BEI	Baroreflex effectiveness index
BMI	Body mass index
(o)BP	(Office) Blood pressure
BPV	Blood pressure variability
BRS	Baroreflex sensitivity
BRSA	Baroreflex sensitivity - Area method
BRST	Baroreflex sensitivity - Threshold method
CB	Carotid body
CBF	Cerebral blood flow
cBRS	Cardiac baroreflex sensitivity
CCB	Calcium channel blocker
CHD	Coronary heart disease
CHF	Congestive heart failure
CKD	Chronic kidney disease
CMR	Cardiac magnetic resonance imaging

CNS	Central nervous system
CO	Cardiac output
CO2	Carbon dioxide
CoW	Circle of Willis
CRP	C reactive protein
CT	Computerised tomography
CVD	Cardiovascular disease
CVLM	Caudal ventrolateral medulla
CVR	Cerebrovascular resistance
(o)DBP	(Office) Diastolic blood pressure
DBS	Deep brain stimulation
ECG	Electrocardiogram
ECV	Extracellular volume fraction
EDV	End diastolic volume
eGFR	Estimated glomerular filtration rate
ENaC	Epithelial sodium channel
ESV	End systolic volume
ETCO2	End-tidal carbon dioxide
FiO2	Fraction of inspired oxygen
HBPM	Home blood pressure monitoring
(n)HF	(Normalised) high frequency spectral power
HR	Heart rate
HRV	Heart rate variability
HTN	Hypertension
HVR	Hypoxic ventilatory response
IL	Interleukin
IPV	Instantaneous peak velocity
ISH	Isolated systolic hypertension
IV	Interstitial volume
(n)LF	(Normalised) low frequency spectral power

LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVM(i)	Left ventricular mass (indexed to body surface area)
(o)MAP	(Office) Mean arterial pressure
MCV	Myocardial cell volume
MPO	Myeloperoxidase
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MSNA	Muscle sympathetic nerve activity
MV	Minute ventilation
N2	Nitrogen
Na^+	Sodium ion
NA	Noradrenaline
NF-κβ	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
(p)NN50	(Percentage) successive NN intervals measuring >50 ms
NT-proBNP	N-terminal pro-brain natriuretic peptide
NTS	Nucleus of the solitary tract
OSA	Obstructive sleep apnoea
(o)PP	(Office) Pulse pressure
pcBRS	Pharmacological cardiac baroreflex sensitivity
psBRS	Pharmacological sympathetic baroreflex sensitivity
PVN	Paraventricular nucleus
PVR	Peripheral vascular resistance
PWV	Pulse wave velocity
RA	Renal artery
RAAS	Renin angiotensin aldosterone system
RAS	Renal artery stenosis

RBF	Renal blood flow
RCT	Randomised controlled trial
RDN	Renal denervation
RF	Radiofrequency
RFR	Renal flow reserve
rHTN	Resistant hypertension
RMSSD	Square root of mean squared differences of successive normal-to-normal intervals
RNS	Renal nerve stimulation
RRI	R wave-to-R wave interval
RRI	Renal resistive index
RVLM	Rostral ventrolateral medulla
(o)SBP	(Office) Systolic blood pressure
sBRS	Sympathetic (vasomotor) baroreflex sensitivity
scBRS	Spontaneous cardiac baroreflex sensitivity
SDNN	Standard deviation of normal-to-normal intervals
SHR	Spontaneous hypertensive rat
SNA	Sympathetic nerve activity
SNS	Sympathetic nervous system
SpO2	Blood oxygen saturation
ssBRS	Spontaneous sympathetic baroreflex sensitivity
SV	Stroke volume
TNFα	Tumour necrosis factor alpha
TPR	Total peripheral resistance
VAH	Vertebral artery hypoplasia
VLF	Very low frequency spectral power
VT	Ventricular tachycardia
WDE	Whole dose equivalent

1 Introduction

Hypertension is a global health problem with world-wide prevalence predicted to rise to 1.56 billion by 2025 (Kearney, Whelton et al. 2005). In England approximately 30% of the adult population has hypertension, but blood pressure (BP) remains uncontrolled in over 40% of those receiving treatment (Foundation 2017). These patients have treatment resistant hypertension, which is defined as the failure to achieve a BP of <140/90 mmHg despite compliance with ≥3 anti-hypertensive medications including a diuretic. There are many causes of resistant hypertension, but differentiating between patients with true drug resistance, as opposed to those with pseudo-resistance due to factors such as poor medication adherence, drug intolerance or secondary hypertension is essential. Hypertension is a significant risk factor for cardiovascular disease with the World Health Organisation reporting that 11% of all disease burden in developed countries is due to high BP (WHO 2013), and financial estimates indicate that if BP could be reduced to less than 140/90mmHg, the NHS could save around £97.2 million from reduced complications such as stroke, heart failure and renal failure (Lloyd, Schmieder et al. 2003).

Previously, the treatment options for patients with drug resistant hypertension were very limited, but the advent of novel interventional therapies such as renal denervation (RDN) has generated considerable interest. RDN is an endovascular ablation technique which aims to disrupt the afferent and efferent renal nerves, thereby abolishing a feedback loop which drives up sympathetic nerve activity (SNA) and hypertension. Initial proof of concept and safety studies (Symplicity HTN-1 and EnligHTN I) and a subsequent randomised controlled trial (RCT, Symplicity HTN-2) reported response (≥10 mmHg drop in office systolic blood pressure (oSBP)) rates of ≥80% at 6 months following RDN (Krum, Schlaich et al. 2009, Esler, Krum et al. 2010, Worthley, Tsioufis et al. 2013). Significant reductions in office BP were maintained out to at least 24 months after denervation in all three of these studies (-29/-14 mmHg, -29/-13 mmHg and -30/-11 mmHg respectively) (Esler, Bohm et al. 2014, Krum, Schlaich et al. 2014, Tsioufis, Papademetriou et al. 2015). These initial finding fuelled a huge acceleration of research in the field, but did not reflect data from other European groups reporting response rates of closer to 50%, with considerable variability in the BP outcomes post-RDN between individual patients (Brinkmann, Heusser et al. 2012, Prochnau, Lucas et al. 2012, Vase, Mathiassen et al. 2012, Kaltenbach, Franke et al. 2013). It was in this context that this pilot study was designed, aiming to investigate factors which would predict whether an individual would response to this expensive and invasive new technique.

Two years into this project, the outcomes of the American sham-RCT (Symplicity HTN-3) were published and this pivotal study failed to meet its primary outcome of a reduction in office BP at 6 months, prompting renewed discussion into the efficacy of RDN (Bhatt, Kandzari et al. 2014). The mechanisms underlying the effect of RDN remain unclear and given the significant variability in the BP response to RDN between studies, generating an individual patient's autonomic profile and relating this to their treatment outcome should help to guide patient selection for this invasive therapy. There has been considerable debate about the optimal study design and outcome measures to be used when assessing the efficacy of novel treatments of hypertension, and indeed whether RDN has any clinical antihypertensive effect at all (Esler 2014, Mahfoud, Edelman et al.

2014, Kandzari, Bhatt et al. 2015, Lobo, de Belder et al. 2015, Gulati, Raphael et al. 2016, Howard, Shun-Shin et al. 2016).

If RDN is effective in reducing blood pressure, then why are individual outcomes so variable? If a patient fails to respond to RDN is it because the procedure itself was not effective at ablating the renal nerves, or is it because hypertension in that individual is driven by factors other than raised sympathetic nerve activity, and that a therapy targeting this mechanism was never going to be beneficial in that particular patient? It is the latter question that this study aims to address through measures of the efficacy of renal denervation at the time of the procedure, and through comprehensive autonomic profiling of participants before and after RDN with a view to establishing physiological predictors of response for this novel, interventional therapy for the management of resistant hypertension.

We measured a range of physiological parameters in patients before and after RDN, including muscle sympathetic nerve activity (MSNA) using a technique called microneurography, as well as heart rate variability, sympathovascular transduction, baroreflex sensitivity and chemoreflex sensitivity, markers of inflammation, cerebral blood flow, and measures of left ventricular function and mass and aortic distensibility. These indices will be correlated against changes in office BP following denervation, and we hypothesise that this will facilitate the identification of those most likely to response to RDN. We also aimed to develop measures of technical efficacy for RDN by examining its effect on reflex responses to stimulation of both the afferent and efferent renal nerves, thus enabling the appropriate interpretation of BP outcomes and helping to direct the development of future generations of ablation catheters.

Our data are presented in the context of the highs and lows seen in the field of renal denervation over the course of this study, and look to the future of this exciting, but at times controversial, new technique.

2 Literature review

2.1 Blood pressure regulation

2.1.1 The role of the kidney in blood pressure regulation

The kidney has long been established as central in the regulation of blood pressure. As early as the 19th century, Bright proposed that alterations in urine production by the kidney were associated with changes in the blood which caused increases in vascular resistance, and thus increased blood pressure and cardiac mass (Bright 1836). In 1909, Starling discussed the interdependence of fluid balance and circulatory stability, and the regulatory function of the kidney; the heart responds to the volume of the circulation and the kidneys adjust the volume of excretion (Starling 1909). These concepts were developed by Guyton and Coleman in the 1960s to form the Guytonian (or more correctly Coleman-Guytonian) Paradigm, the 'renal – body fluid feedback control system', in which the kidney underpins blood pressure control (Guyton 1961, Guyton and Coleman 1969, Guyton 1989).

2.1.1.1 The Coleman-Guytonian Paradigm

The renal – body fluid control system establishes several core concepts for the long-term control of blood pressure (Guyton and Hall 1996, Brands 2012, Pao 2014, Evans and Bie 2016, Osborn and Foss 2017).

1. Increased extracellular fluid volume causes increased arterial pressure, which has a direct effect on the kidney, increasing sodium ion (and water) filtration and excretion known as pressure natriuresis.
2. The point at which sodium ion (Na^+) intake intersects with the renal sodium handling/function curve determines the blood pressure set point (Figure 2-1).
3. There is infinite gain within the pressure natriuresis system which will return blood pressure to its equilibrium point. To change the blood pressure set point, there must either be an increase in salt intake or a shift in the renal function curve (deteriorating renal function and thus Na^+ excretion would shift the curve to the right, increasing the set point of arterial pressure).
4. Blood flow is precisely controlled to each tissue (autoregulation); cardiac output is the sum of blood flow to all tissues.
5. Arterial pressure is kept constant, independent of cardiac output. This prevents changes in blood flow in one organ affecting flow elsewhere in the body as the pressure head remains constant.
6. According to Ohm's law, pressure = flow x resistance. However, changes in systemic vascular resistance do not increase the long-term blood pressure set point, since the initial increase in arterial pressure related to increased systemic vascular resistance drives pressure-natriuresis, returning blood pressure to normal.

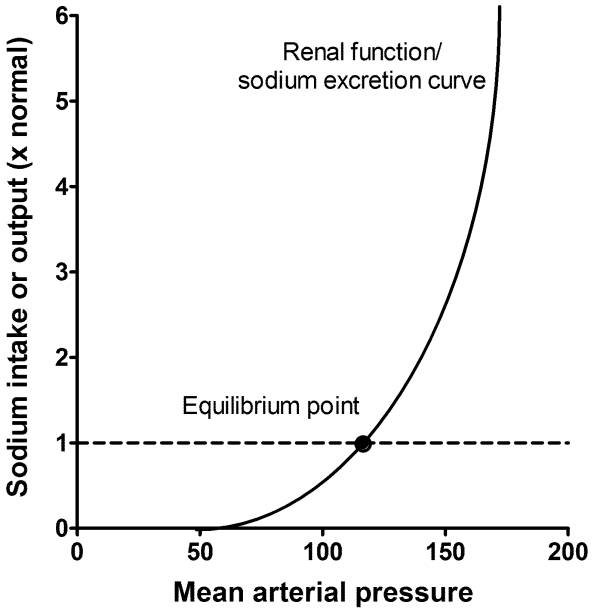


Figure 2-1. Pressure natriuresis curve

Whilst pressure natriuresis is the main tenet of the renal – body fluid system, there are other neurohormonal mechanisms which influence the renal control of blood pressure. The renin angiotensin aldosterone system (RAAS) is the predominant of these pathways, with a key role in maintaining blood pressure homeostasis despite fluctuations in salt intake (Guyton and Hall 1996); important given that a high salt diet does not cause hypertension in all cases. The RAAS pathway is summarised in Figure 2-2.

Renin is released from the juxtaglomerular cells in the kidney in response to a fall in arterial pressure, which leads to an increase in the circulating levels of highly potent angiotensin II (Ang II). Ang II acts on the kidney to constrict the efferent renal arteriole, increasing glomerular filtration, but acts to increase Na^+ and water reabsorption in the renal tubules, resulting in net Na+ and water retention. Ang II resets the arterial baroreflex, activating the baroreceptors and reducing sympathetic nerve activity (SNA), acting to buffer increases in blood pressure in response to Ang II (Lohmeier and Iliescu 2015). Ang II also stimulates aldosterone release from the zona glomerulosa of the adrenal gland by action on angiotensin II type 1 receptors (AT1R) (Hall 1986, Guyton and Hall 1996, Coffman 2014). Aldosterone works through activation of the mineralocorticoid receptor and consequent assembly and translocation of the epithelial sodium channel (ENaC) which facilitates sodium reabsorption in the distal renal tubules, as well as having a role in regulating water absorption by decreasing aquaporin expression in the collecting ducts (Guyton and Hall 1996, Nielsen, Kwon et al. 2006, Coffman 2014). Well known antihypertensive medications, including angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB), direct renin inhibitors (aliskiren) and aldosterone antagonists, all act to block the rise in blood pressure triggered by RAAS activation.

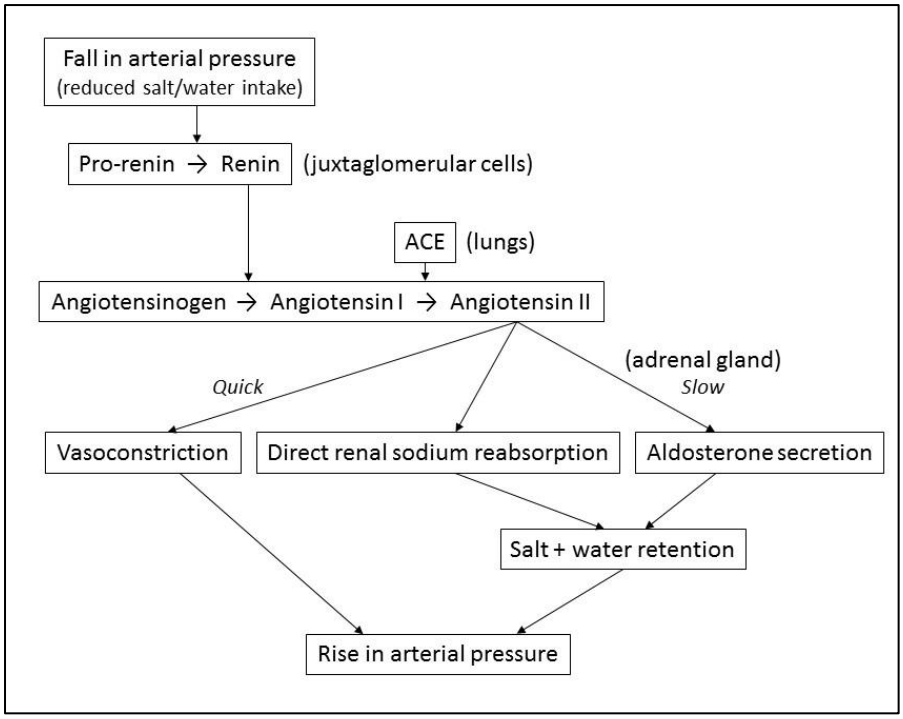


Figure 2-2. Renin angiotensin aldosterone system
ACE; angiotensin converting enzyme.

The RAAS pathway takes the Guytonian paradigm beyond simple pressure natriuresis, but does not contradict it, since the RAAS pathway also responds to falls in arterial pressure and thus extracellular fluid volume with an increase in sodium reabsorption and a consequent rise in pressure.

The sympathetic nervous system also has a synergistic role to play in blood pressure control via the kidney. Increased renal sympathetic nerve activity causes constriction of the renal arterioles, resulting in reduced renal blood flow and glomerular filtration, increased sodium reabsorption in the Loop of Henle, and increased renin and Ang II formation (Guyton and Hall 1996). Stimulation of the β-adrenoreceptors causes macula densa cells to increase renin release (Holdaas, DiBona et al. 1981), and also increases blood pressure by suppressing WNK4 (with no lysine kinase 4) and, in turn, enhancing sodium chloride cotransporter (NCC) activity and hence sodium reabsorption (Mu, Shimosawa et al. 2011).

2.1.1.2 Neural mechanisms for the short-term regulation of blood pressure

Whilst the kidney plays a central role in the long term regulation of blood pressure and contributes to the chronic set point for blood pressure, Guyton highlights several other key mechanisms which help the body to maintain a constant blood pressure in response to physiological challenges in the short term (Guyton and Hall 1996). We have already considered the RAAS system which adapts to changes in blood pressure over the short to medium term, fluctuating over minutes and hours, but there are a range of reflexes which respond to acute changes in pressure, such as changes in posture or acute blood/volume loss (Guyton and Hall 1996). These short-term mechanisms primarily act through modulation of the sympathetic nervous system, with parasympathetic activity via the vagal nerve mainly limited to reflex changes in heart rate (Guyton and Hall 1996).

The central integration of the reflexes and their control of sympathetic outflow is summarised in Figure 2-3.

The vasomotor neural network is located within the brainstem at the level of the medulla oblongata. The centre consists of:

i. a sympathoexcitatory area located in the rostral ventrolateral medulla (RVLM). These neurons contain VGLUT2 (vesicular glutamate transporter 2) and tyrosine hydroxylase and send axons to the spinal cord and are therefore termed bulbospinal pre-sympathetic motoneurons (Guyenet 2014).They innervate the sympathetic preganglionic neurons located in the intermediolateral cell column of the spinal cord that via the postganglionic sympathetic neurons innervate the heart, arterioles and venules (Guyenet 2006). The adrenal medulla is innervated directly by the preganglionic neurons for the secretion of adrenaline and noradrenaline into the blood (Guyton and Hall 1996, Fisher and Paton 2012).
ii. a sympthoinhibitory area located in caudal ventrolateral medulla (CVLM). The CVLM contains neurons containing GABA (γ- aminobutyric acid) that project to and terminate on the RVLM bulbospinal pre-sympathetic neurons and have an inhibitory effect, thereby reducing heart rate, total peripheral resistance and venous pressure (Guyton and Hall 1996, Guyenet 2006, Fisher and Paton 2012).
iii. a sensory area in the nucleus of the solitary tract (NTS) in the dorsomedial medulla receives sensory signals from the vagus and glossopharyngeal nerves (including signals from the baroreceptors and peripheral chemoreceptors) and acts to control the CVLM and RVLM; providing reflex control of circulatory function (Guyton and Hall 1996, Guyenet 2006, Fisher and Paton 2012).

The RVLM pre-sympathetic neurons transmit continuous signals resulting in sympathetic vasomotor tone (Guyton and Hall 1996, Guyenet 2006). The significance of this has been demonstrated in animal studies since a fall in arterial pressure is seen following total spinal anaesthesia and blockade of the sympathetic vasoconstrictor neurones (Guyton and Hall 1996).

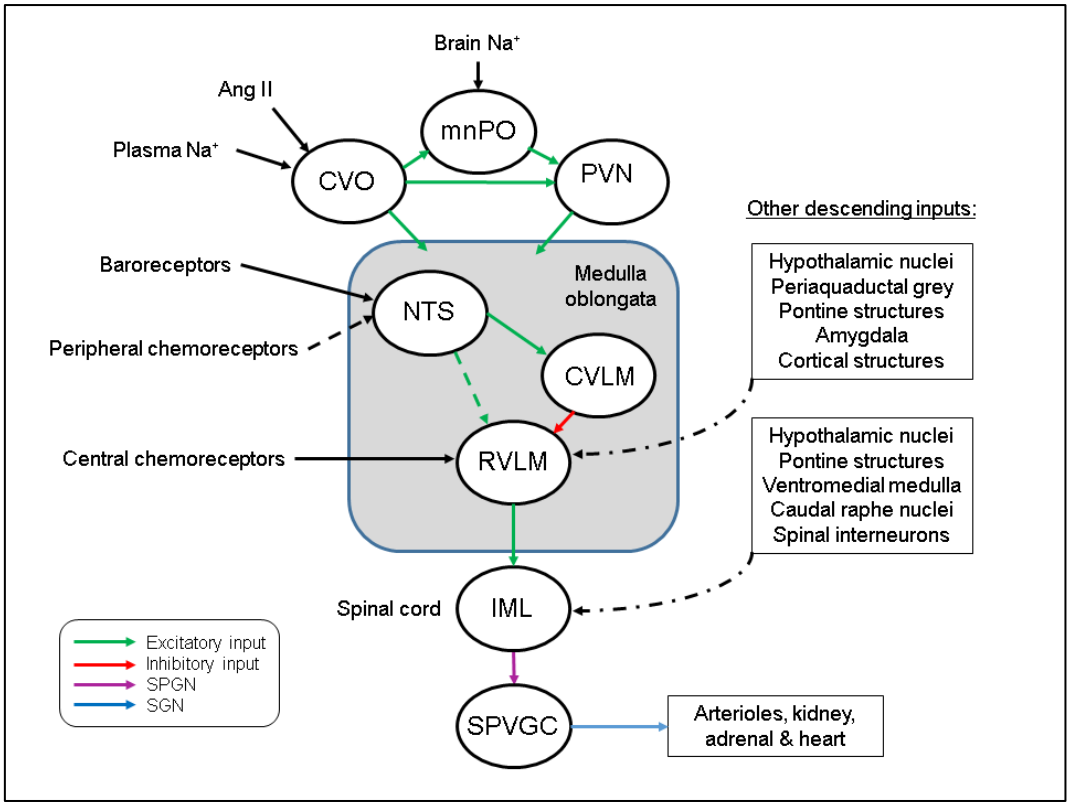


Figure 2-3. Central neural sites involved in the integration of cardiovascular reflexes and the control of sympathetic outflow.
Chemoreceptor circuit is shown with dashed lines to differentiate from the baroreceptor circuit; the chemo circuit inputs directly from the NTS to the RVLM. CVLM, caudal ventrolateral medulla; CVO, circumventricular organs; IML, intermediolateral cell column; mnPO, median preoptic nucleus; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus; RVLM, rostral ventrolateral medulla; SGN, sympathetic ganglionic neuron; SPGN, sympathetic preganglionic neuron, SPVGC, sympathetic paravertebral ganglionic chain. Adapted from Fisher & Paton 2012 and Guyenet 2006 (Guyenet 2006, Fisher and Paton 2012).

Stretch receptors in the aorta and carotid sinus are the origin of the sensory afferent components of the *arterial baroreflex*. This highly sensitive system has evolved as a homeostatic mechanism enabling us to adapt to changes in posture, immersion in water and volume loss due to haemorrhage or dehydration. The arterial baroreflex is activated between 60-180 mmHg, with maximal sensitivity in the normal operating range of 100 mmHg (Guyton and Hall 1996). Distension as a result of increased intravascular pressure activates this sympathoinhibitory reflex; the signal from the baroreceptors is transmitted via the NTS and has an excitatory effect on the CVLM which in turn inhibits the RVLM sympathoexcitatory neurons resulting in systemic and venous vasodilation and negative chronotropic and inotropic effects on the heart (Guyton and Hall 1996, Guyenet 2006). The baroreflex is crucial in the control of beat-to-beat changes in blood pressure, but resets to a new operating blood pressure over 1-2 days. This was demonstrated in experiments by Cowley in the 1970s, in which complete surgical disruption of the arterial baroreceptors produced only a transient elevation in BP in awake dogs (Cowley 1992). The baroreflex was not felt by Guyton to have a role in the

long-term control of blood pressure, although this perspective has been brought into question more recently by evidence from Lohmeier and Thrasher for a long-term role for the baroreflex in BP regulation (see Section 2.1.2.3.2) (Guyton and Hall 1996, Thrasher 2002, Malpas 2010, Lohmeier and Iliescu 2015).

The *peripheral chemoreceptors* trigger respiratory drive and sympathetically mediated reflex increases in BP when stimulated with hypoxia and/or hypercapnia (or acidic pH) (Paton, Sobotka et al. 2013). They are located bilaterally at the carotid bifurcation (carotid bodies), and the aorta (aortic bodies) (Paton, Sobotka et al. 2013) and excite carotid sinus and aortic nerve fibres respectively that terminate in the NTS. When blood pressure falls these highly sensitive organs are subject to decreased oxygen and increased carbon dioxide tension (due to reduce blood flow) and trigger reflex increases in sympathetic activity to increase arterial pressure (Guyton and Hall 1996).

Right atrial and pulmonary low pressure receptors primarily respond to changes in blood volume in the low pressure circulation (Paintal 1973, Guyton and Hall 1996), for example, an increase in right atrial pressure causes an increase in heart rate and reduction in vasomotor sympathetic activity reducing total peripheral resistance known as the Bainbridge reflex (Jones 1962). In addition to this neural mechanism, *atrial natriuretic peptide* (ANP) is released from cardiac muscle cells in response to overstretching of the atria, therefore acting as a parallel response to volume overload by increasing glomerular filtration rate, reducing Na^+ reabsorption in the distal renal tubule and collecting duct, decreasing renin release, and by causing vasodilatation of the arterioles and venules (Ballermann and Brenner 1987, Guyton and Hall 1996). Conversely, when there is a fall in extracellular volume with an increase in osmolarity, the osmoreceptor cells in the anterior hypothalamus are stimulated, triggering release of *vasopressin* (also known as anti-diuretic hormone (ADH)) from the posterior pituitary. Vasopressin , increases the water permeability of the distal renal tubules and collecting ducts, increasing water reabsorption to maintain volume (Guyton and Hall 1996).

Guyton describes the *central nervous system (CNS) ischaemic response*, which activates an at arterial pressure of <60 mmHg, as the final defence mechanism against cerebral hypoperfusion (Sagawa, Ross et al. 1961, Guyton and Hall 1996); if blood flow to the brainstem drops sufficiently to cause ischaemia, neurons within the RVLM are directly excited and act to dramatically increase blood pressure through intense peripheral vasoconstriction (Koganezawa and Paton 2014). Initially described by Harvey Cushing in 1901, the Cushing response is a specific type of CNS ischaemic response which occurs when blood flow to the vasomotor centre is restricted due to increased pressure within the cranial vault (Cushing 1901). More recently, Paton et al. have argued that the *Cushing's response* lies the extreme end of a more sensitive *Cushing's mechanism*, which responds to changes in changes in cerebral perfusion at normal physiological levels of arterial pressure, although this is yet to be validated (Paton, Dickinson et al. 2009).

2.1.1.3 How does the Guytonian Paradigm explain hypertension?

Guyton describes the condition of volume loading hypertension (Guyton and Hall 1996). On a simplistic level, fluid retention and increased extracellular volume will cause an initial increase in cardiac output and blood pressure. Total peripheral vascular resistance then increases in response to this to protect target organs from hyperperfusion through

the mechanism of 'whole-body autoregulation'[1]. Left heart cardiac output (the sum of blood flow to all tissues) thus returns to normal, but blood pressure remains high due to the sustained increase in total peripheral vascular resistance (PVR). Thus, increased PVR can maintain hypertension but does not cause it without an initial increase extracellular fluid volume due to sodium (or volume) loading. However, this raises questions; in the short term, where does the excess volume go (a shift into capacitance vessels would contradict the increase in PVR), and why does BP not return to normal if the increased pressure causes a natriuresis/diuresis (the natriuresis curve would have to shift if hypertension is sustained)? When blood pressure increases, the short-term pressure regulatory mechanisms described in Section 2.1.1.2 must also reset to operate over the higher BP range. For example, regardless of whether it is involved in the long-term regulation of BP or not, the baroreflex must have a rightward shift to reset over a higher pressure range.

Conversely, pathological renal hypoperfusion can also cause hypertension. In the Goldblatt one-kidney, one-clip model, there is an initial increase in arterial pressure related to renin release and activation of the RAAS, and a sustained increase in pressure due to salt retention and volume expansion resulting in renovascular hypertension (Liard, Cowley et al. 1974). Renal artery stenosis also results in a relative reduction in renal blood flow; this stimulates increased renin release from the juxtaglomerular cells and activation of the RAAS, including action of renin and Ang II on the contralateral kidney (Guyton and Hall 1996). Reduced renal blood flow will also cause a shift of the pressure-natriuresis curve and therefore promote increased sodium reabsorption and inappropriate extracellular volume expansion because of pathological renal hypoperfusion.

It is intuitive to understand how impaired renal function can cause hypertension based on the renal – body fluid system. In chronic kidney disease patients develop patchy renal ischaemia which once again drives renin release and activation of the RAAS, as well as a right-ward shift in the renal function/sodium handling curve (Guyton and Hall 1996).

2.1.1.4 Evidence to support the central role of the kidney in hypertension

Liddle's syndrome is a classic example of the way in which changes in renal salt handling, in this case increased sodium reabsorption, result in an increase in blood pressure. Individuals with this condition have a gain of function mutation in the epithelial sodium channel (ENaC) which acts to increase sodium reabsorption, thus shifting the set point of the pressure natriuresis curve to a higher arterial pressure (Pao 2014). Blood pressure control can be achieved in patients with Liddle's syndrome following treatment with triamterene, an ENaC-specific inhibitor, and a low salt diet (Botero-Velez, Curtis et al. 1994, Pao 2014).

[1] Autoregulation of blood has both metabolic (through regulation of local vasodilatory substances, such as adenosine and nitric oxide, in response to local tissue oxygenation and nutrient supply) and myogenic mechanisms (with the sudden stretch of small blood vessels causing the smooth muscle of the vessel wall to contract).

Cross-transplantation studies provide evidence that hypertension tracks intrinsic kidney function (Pao 2014). For example transplantation of the kidneys from a Dahl salt-sensitive rat into a normotensive salt-resistant rat results in hypertension in the normotensive animal, regardless of whether transplantation occurs before or after the introduction of a high-salt diet (Dahl, Heine et al. 1974). The converse is also true, with transplantation of kidneys from a normotensive donor animal into a hypertensive recipient blunting the hypertensive propensity of the strain (Dahl, Heine et al. 1974). The same phenomenon is observed following renal transplantation in hypertensive humans receiving graphs from normotensive donors (Curtis, Luke et al. 1983).

Transgenic transplantation studies have also demonstrated the key role for local RAAS feedback within the kidney. Hall et al. had previously demonstrated that intra-renal infusion of Ang II increases sodium reabsorption, shifting the renal function/sodium handling curve to the right, and thereby causing hypertension (Hall 1986). Coffman's group transplanted kidneys from AT1R knockout mice into normal recipients expressing systemic AT1R (and vice versa), demonstrating that expression of the AT1R in the kidney is necessary and sufficient for the development of Ang II induced hypertension (Crowley, Gurley et al. 2006), although the important central effect of Ang II must also be considered (see section 2.1.2.3.6).

Importantly for this research project, denervation of the kidney has been shown to reduce blood pressure in both animal and human studies (Liard 1977, Katholi, Winternitz et al. 1982, Lee and Walsh 1983, Esler, Krum et al. 2010, Schlaich, Bart et al. 2013). This demonstrates the central role of the kidney in the neural regulation of blood pressure, but whether disruption of efferent sympathetic signals to the kidney, or sensory afferent signals from the kidney providing central feedback, underpins this anti-hypertensive effect is still unknown (Grassi, Mark et al. 2015). The data supporting renal denervation will be discussed in detail in Section 2.3.

2.1.1.5 Hypertension: Going beyond Guyton

Guyton's renal – body fluid control hypothesis provides a robust explanation for blood pressure control; however, it does not fit all data, particularly mechanisms effective beyond the kidney, and other factors should be considered. Importantly, the paradigm does not include the autonomic nervous system – where does sympathetic nerve activity fit in?

Extra-renal angiotensin II type 1a receptors, most likely those located within the brain and vasculature, have a role to play in blood pressure regulation (Pao 2014). Further data from Coffman's group using cross-transplantation between wild-type and AT1R knockout mice found that AT1R knockout mice without renal or peripheral angiotensin II type 1 receptors are hypotensive, but that transplantation of a wild-type AT1R+ kidney into an AT1R knockout recipient was not sufficient to increase the blood pressure to that of AT1R+ wild-type controls with both renal and peripheral angiotensin II type 1 receptors (Crowley, Gurley et al. 2005). In vivo gene transfer studies have also demonstrated that Ang II can act via endothelial nitric oxide synthase (eNOS) to release nitric oxide from the vasculature within the NTS, which then modulates the baroreflex through vascular neuronal signalling (Paton, Waki et al. 2003, Paton, Wang et al. 2008).

Guyton hypothesised that hypertension does not occur due to a primary increase in total peripheral resistance (TPR), and that any increase in TPR is secondary to whole body autoregulation in response to sodium/volume loading (Guyton 1989). However, there is increasing evidence that primary increases in TPR can cause hypertension (Pao 2014), for example through the action of aldosterone on the mineralocorticoid receptor in vascular smooth muscle, which causes vasoconstriction. McCurley et al. reported that transgenic mice with an inducible deletion of the mineralocorticoid receptor in vascular smooth muscle did not have the age-related increase in arterial pressure seen in wild-type mice but showed no difference in renal sodium handling to their wild-type counterparts (McCurley, Pires et al. 2012). Likewise, mice with a constitutively inactive mineralocorticoid receptor in the vascular smooth muscle endothelium, demonstrated normal sodium excretion, but were hypotensive with attenuated aldosterone-induced vascular stiffness (Galmiche, Pizard et al. 2014).

The renal-body fluid hypothesis as applied to renovascular hypertension and the Goldblatt model described above must also be reconsidered. Oliveira-Sales et al. have shown that sympathetic nerve activity is raised prior to the development of hypertension in a rat two-kidney, one-clip Goldblatt model (Oliveira-Sales, Colombari et al. 2016). Furthermore, elevated superoxide levels in the RVLM are required for the development and maintenance of one-kidney, one-clip hypertension, emphasing the risks of over-simplification (Oliveira-Sales, Colombari et al. 2010).

Sodium homeostasis may also be more complex than represented by the Guytonian paradigm. Guyton's hypothesis is based on the concept that the two components of extracellular fluid volume, intravascular and interstitial fluid, are in equilibrium (Coffman 2014). However, work by Titze's group suggests that the interstitium of the skin may act as a sodium reservoir, buffering the impact of sodium accumulation on intravascular volume and arterial pressure (Machnik, Neuhofer et al. 2009). This is of particular relevance in understanding the mechanisms driving salt-sensitive hypertension, in which individuals demonstrate an exaggerated blood pressure effect in response to changes in salt intake, which may not solely relate to alterations in sodium handling.

There is increasing evidence that the immune system has a key role to pay in the development of hypertension. Disruption of T cell function, particularly T cell activation, is protective against the development of Ang II mediated hypertension (Guzik, Hoch et al. 2007, Vinh, Chen et al. 2010). Furthermore, whilst the development of hypertension maybe dependent on CD8+ T cells (Trott, Thabet et al. 2014), CD4+ T cells may be relevant to sustained hypertension, with Th17 cells (secreting IL-17) and Th1 cells being implicated in the potentiation of hypertension (Madhur, Lob et al. 2010) and hypertension related kidney injury (Zhang, Patel et al. 2012), respectively. The NF-κβ (nuclear factor kappa-light-chain-enhancer of activated B cells) cascade is a pro-inflammatory pathway which mobilises inflammatory cytokines and reactive oxygen species; blockade of NF-κβ signalling protects against hypertension (Muller, Dechend et al. 2000), and interestingly, central NF-κβ activation by Ang II in the paraventricular nucleus of the hypothalamus has been shown to increase sympathetic nerve activity (Kang, Ma et al. 2009). Systemic inflammation can upregulate microglia, pro-inflammatory cytokines and reactive oxygen species within the rostral ventrolateral medulla, with an associated rise in blood pressure and increased sympathetic vasomotor tone (Wu, Chan et al. 2012). A range of inflammatory cytokines have been implicated in

hypertension, for example, knockouts for the interferon-γ receptor are protected against end organ damage in hypertension (Marko, Kvakan et al. 2012), and interleukin-6 knockout mice have a blunted hypertensive response to Ang II (Lee, Sturgis et al. 2006). Some consideration must be attended to the Ang II model of hypertension, which is far from realism, since homeostatic responses to an increase in Ang II would usually act to return the activation of the RAAS system to resting levels. These animal studies indicate that immune modulation may represent an exciting novel target for the management of hypertension, and whilst this is supported by preliminary clinical data (Herrera, Ferrebuz et al. 2006), this is a field that will benefit from further clinical research.

The selfish brain hypothesis places the brain, rather than the kidney, at the centre of blood pressure control, and is based on the concept that the brainstem will maintain adequate perfusion and oxygenation at the expense of systemic hypertension, hence 'essential hypertension' (Cates, Dickinson et al. 2012). Patients with hypertension have relative narrowing of the vertebral arteries (Dickinson and Thomson 1960) and raised SNA (Smith, Graham et al. 2004), and there is evidence to suggest that cerebro-hypoperfusion drives sympathoexcitation (Cates, Dickinson et al. 2012). It is observed that sympathetic overdrive may fuel the pathogenesis of hypertension, with a progressive increase in SNA from normal to mild-moderate hypertension and then severely hypertensive patients, corroborating findings in the animal models of hypertension (Grassi, Seravalle et al. 2010). Fisher and Paton propose a paradigm relating raised SNA with inflammation, cerebral hypoperfusion and angiotensin II activity in the aetiology of hypertension (see Figure 2-4) (Fisher and Paton 2012); this concept of 'neurogenic hypertension' will be explored in the following section.

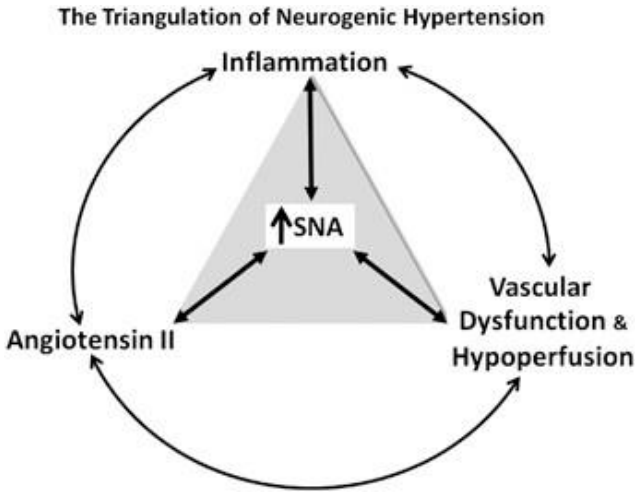


Figure 2-4. Triangulation of neurogenic hypertension
The establishment of positive feedback loops between angiotensin II, inflammation and vascular dysfunction/brain hypoperfusion may form the basis of refractory hypertension (Fisher and Paton 2012).

2.1.2 Neurogenic hypertension

The pathogenesis of hypertension is highly complex and due to a combination of genetic and environmental factors, the severity of which will vary between individuals. In ~95% of cases there is no clear aetiology, and the diagnosis of exclusion – 'primary (or essential) hypertension' - is given (Carretero and Oparil 2000). The genes responsible for certain monogenetic hypertensive syndromes (e.g. glucocorticoid-remediable aldosteronism, Liddle's syndrome, and apparent mineralocorticoid excess) have been identified (Carretero and Oparil 2000, Lifton, Gharavi et al. 2001), but these conditions are rare, and family and twin studies suggest that in the majority blood pressure is a classic complex genetic trait with a heritability of 30-50% (Ehret and Caulfield 2013). Genome-wide analysis has now identified at least 43 genetic variants associated with systolic blood pressure, diastolic blood pressure and hypertension, but it is likely that hundreds of loci are involved, and the effect size of each of the identified variants is only ~1 mmHg of systolic BP (Ehret and Caulfield 2013). Environmental and physiological factors that increase blood pressure include aging, obesity, insulin resistance, high alcohol intake, high salt intake (in salt-sensitive patients), stress and a sedentary life-style; several of these are also partially genetically determined, thus complicating the picture even further (Carretero and Oparil 2000).

On a physiological level, blood pressure is the product of cardiac output (CO) and peripheral vascular resistance (PVR). These parameters are controlled by intermediary mechanisms including the sympathetic nervous system (SNS), parasympathetic nervous system (heart rate), renin-angiotensin-aldosterone system, renal kallikrein-kinin systems and endothelial factors, which in turn influence sodium excretion, vascular reactivity, and cardiac contractility and respond to blood pressure feedback (Carretero and Oparil 2000). The 1967 Guyton and Coleman model for the control of blood pressure directly relating changes in mean arterial pressure and kidney perfusion to renal sodium and water excretion has now been expanded to incorporate the dynamic role of the SNS in long-term blood pressure regulation (Osborn, Averina et al. 2009). Hypertension can be regarded as neurogenic if it is due to an abnormality of the autonomic nervous system, as opposed to being caused by a primary vascular, renal, or specific endocrine abnormality (Guyenet 2006)

Historically, centrally acting sympatholytic agents (clonidine, methyldopa) have often been poorly tolerated, and the majority of pharmacological research in the field has targeted the RAAS (ACE inhibitors/ARB), salt and water retention (diuretics) or increased peripheral vascular tone and myocardial contractility (calcium channel blockers, beta blockers, alpha blockers). However, the pivotal role of the SNS in neurogenic hypertension is now coming to the fore, and the relationship between excessive SNA and the development and progression of hypertension, insulin resistance, chronic renal disease and heart failure has been demonstrated in both preclinical and human experiments (Goldstein 1983, Cohn, Levine et al. 1984, Hasking, Esler et al. 1986, Mancia, Grassi et al. 1999, Hausberg, Kosch et al. 2002, Penne, Neumann et al. 2009, Grassi, Quarti-Trevano et al. 2011, Sobotka, Mahfoud et al. 2011, Schlaich, Hering et al. 2012). Exactly why sympathetic up-regulation occurs and how to most reliably measure it will form the subject of this review, with particular reference to the roles of the renal afferent and efferent nerves in the development and maintenance of neurogenic hypertension.

2.1.2.1 Sympathetic overdrive in hypertension

A causal relationship between raised SNA and hypertension is suggested by effect of both surgical sympathectomy and sympatholytic drugs (Guyenet 2006). Surgical sympathectomy was used in the 1950s as a treatment for hypertension, successfully lowering blood pressure, but with significant orthostatic side-effects (Whitelaw and Smithwick 1951, Morrissey, Brookes et al. 1953, Longland and Gibb 1954).Sympathetic ganglionic blockade, starting with drugs such as hexamethonium discovered by William Paton in the early 1950s (Paton 1982), was employed to replace surgical sympathectomy, and likewise lowered blood pressure but with significant side effects, including constipation, mydriasis and impotence (Fisher and Paton 2012). Anti-adrenergic drugs, such as beta blockers, peripheral α1 receptor blockers, and centrally acting sympatholytics (α2 receptors blockers and imidoline antagonists) also have a blood pressure lowering effect, and therefore support a role for sympathetic nervous system involvement in hypertension (Fisher and Paton 2012), as do the established antihypertensive agents ACEi (angiotensin converting enzyme inhibitors), ARBs and CCBs (calcium channel blockers) which all decrease sympathetic nerve activity (Niederberger, Aubert et al. 1995).

Evidence for raised SNA in hypertension comes from a wide range of animal and human studies. Plasma catecholamines are frequently elevated in patients with HTN, especially younger patients with established hypertension, which might suggest a role for sympathetic overdrive in the aetiology of hypertension in this group (Goldstein 1983). However, whilst plasma adrenaline and noradrenaline (NA) levels are easily quantified, inter and intra study variability is high and these measures reflect a global marker for systemic rather than organ-specific sympathetic activity and are a blunt tool for the investigation of hypertension (Goldstein 1983). SNA varies between organs, with differential control (Esler, Jennings et al. 1984, Guyenet 2006). Organ specific SNA can be determined using a constant-rate intra-arterial infusion of radiolabelled NA with quantification of venous endogenous NA as measured by isotope dilution, known as 'noradrenaline spillover' (Esler, Jackman et al. 1980, Esler, Jennings et al. 1984, Esler 2010). Systemic and renal NA turnover is increased in hypertensives, particularly in those with obesity related hypertension (Goldstein, Horwitz et al. 1983, Esler, Jennings et al. 1986, Esler and Kaye 2000); however, this is an invasive technique and can't be used for large-scale clinical autonomic phenotyping or for repeated measures to assess long-term treatment effects.

SNA controls vasomotor tone in peripheral blood vessels. In humans, muscle sympathetic nerve activity (MSNA) can be measured directly from the peroneal nerve in the leg using a technique called microneurography. The technique, initially established by Hagbarth, Vallbo, Sundlof and Wallin in the 1960s, is now well validated (Hagbarth and Vallbo 1968, Sundlof and Wallin 1978, Wallin 1978, Wallin and Eckberg 1982, Eckberg, Wallin et al. 1989, Vallbo, Hagbarth et al. 2004, Hart, Charkoudian et al. 2009, Hart, Joyner et al. 2009). Measures of SNA using microneurography correlate well with measures of whole body SNA measured by NA spillover (Wallin, Sundlof et al. 1981, Wallin, Esler et al. 1992). Microneurography has the limitation of measuring an isolated region of sympathetic outflow to one muscle vascular bed, and there are many vascular beds, including the renal and splanchnic circulations, that cannot be accessed using this technique. However, the skeletal muscle is a large vascular bed providing a major

contribution to TPR. Microneurography is also advantaged by its high temporal resolution allowing the dynamic assessment of on-going activity levels and baroreceptor and chemoreceptor reflex sensitivity. MSNA is minimally invasive and can be repeated on multiple occasions to investigate a response to therapy over the longer term, and there is good reproducibility in this measurement for a given individual over time (Yamada, Miyajima et al. 1989, Hart, Joyner et al. 2010). Microneurography can be used to measure both single and multi-unit MSNA, enabling the interpretation of both the central modulation of SNA and the global sympathetic input controlling vasomotor tone within a vascular bed respectively (Hering, Lambert et al. 2013). Single unit measurement of sympathetic neurons (classified as vasoconstrictor neurons by careful consideration of appropriate firing latency and consistent burst morphology), may give additional information about the frequency of post-ganglionic neuron firing, the probability of neuron firing in any particular multi-unit burst, and the occurrence of multiple unit firing in a single burst of activity to a vascular bed, providing more detailed information about the modulation of SNA (Macefield, Wallin et al. 1994). For the purposes of this review and subsequent data analysis, MSNA refers to multi-unit recording which are more widely used and validated.

MSNA is higher in patients with hypertension than in age-matched controls, although MSNA increases with age in both groups (Yamada, Miyajima et al. 1989), but is this increased SNA a consequence raised blood pressure and aging, or does elevated SNA have a role in the aetiology of hypertension? Data from animal models suggests a role for SNA in the development of HTN (Judy, Watanabe et al. 1979, Antic, Kiener-Belforti et al. 2000). Young, normotensive SHR (spontaneously hypertensive rats) have elevated SNA prior to the development of hypertension (Cabassi, Vinci et al. 1998, Simms, Paton et al. 2009), and removing sympathetic and adrenal influences from the SHR in the first 8 weeks of life (sympathectomy and alpha blocker administration) prevents the development of hypertension and left ventricular hypertrophy (LVH) in these animals (Korner, Bobik et al. 1993). Of note, brief treatment with an ACE inhibitor has a similar effect in young SHR, preventing the development of hypertension, and may suggest a central role for Ang II in the development of hypertension in this animal model, illustrating the likely interaction between these different pathologic mechanisms (Harrap, Van der Merwe et al. 1990). SNA has also been shown to be elevated prior to the development of hypertension in rats using a Goldblatt two-kidney, one-clip model (Oliveira-Sales, Colombari et al. 2016).

In humans, MSNA is increased in patients with borderline hypertension when compared against normotensive controls (Anderson, Sinkey et al. 1989), and interestingly, MSNA is higher in those with borderline or early-stage hypertension, and in hypertension with LVH, than in patients with established uncomplicated hypertension, suggesting that it may play a particularly important role in the initiation of the hypertensive state, with persistently high level of SNA potentially conferring a greater risk of target organ damage (Mancia, Grassi et al. 1999, Smith, Graham et al. 2004). MSNA is also increased in white-coat hypertension, although not to the same degree as seen in fulminant disease, consistent with evidence that this is not a completely benign condition (Glen, Elliott et al. 1996, Owens, Lyons et al. 1998, Smith, Graham et al. 2002). The on-going role of SNA in the maintenance of hypertension is illustrated by the administration of centrally acting sympatholytic agents such as moxonidine, which reduce both BP and MSNA in patients with established hypertension (Wenzel, Spieker et al. 1998).

2.1.2.2 Consequences of chronically elevated sympathetic tone

Chronically elevated sympathetic tone causes hypertension by causing vasoconstriction and increasing vascular stiffness. Raised sympathetic outflow impacts upon multiple systems, these changes which often further compound the rise in BP are illustrated in Figure 2-5, and include the well documented association between elevated sympathetic tone and other conditions such as insulin resistance, obstructive sleep apnoea and heart failure (Narkiewicz, van de Borne et al. 1998, Spaak, Egri et al. 2005, Grassi, Seravalle et al. 2010, Sobotka, Mahfoud et al. 2011, Fisher and Paton 2012). Signals transmitted via the efferent renal nerves, which are composed of postganglionic sympathetic fibres, act to increase renin release and sodium retention, and to reduce renal blood flow (RBF) (Winternitz and Oparil 1982, Katholi 1983, DiBona 2005). Increased renal SNA acts predominantly via three different mechanisms; stimulation of β_1-adrenoceptors on juxtaglomerular granular cell triggers increased renin secretion, stimulation of α_{1B}-adrenoceptors on renal tubular epithelial cells causes increased sodium reabsorption, and stimulation of α_{1A}-adrenoceptors on the vascular smooth muscle cells of the afferent and efferent renal arterioles decreases RBF (DiBona 2005). Additionally, sympathetic innervation of the renal pericytes modulates vasoconstriction of the medullary vasa recta through ATP (adenosine triphosphate) release (Crawford, Wildman et al. 2013); contraction of the vasa recta causing a reduction in medullary blood flow, increases Na^+ reabsorption and can drive hypertension (Cowley, Abe et al. 2015).

Patients with hypertension and borderline hypertension also have decreased heart rate variability (HRV), indicating that reduced vagal tone, as well as elevated SNA, contributes to their autonomic imbalance (Liao, Cai et al. 1996, Singh, Larson et al. 1998). Data from groups including the Framingham Study has shown that low HRV is a predictor of both cardiac and all-cause mortality (Tsuji, Larson et al. 1996, Gerritsen, Dekker et al. 2001, Liao, Carnethon et al. 2002). Likewise, sympathetic overdrive with elevated MSNA is associated with end-organ damage, including LVH, congestive cardiac failure, ischaemic cardiac events, arrhythmias and sudden death (Burns, Sivananthan et al. 2007, Grassi, Seravalle et al. 2011, Grassi, Bombelli et al. 2012). Treatments directed at this autonomic pathophysiology may therefore have a significant impact on clinical outcomes, and if the mechanism driving raised SNA in an individual patient can be established, targeted therapy could have an even more beneficial effect.

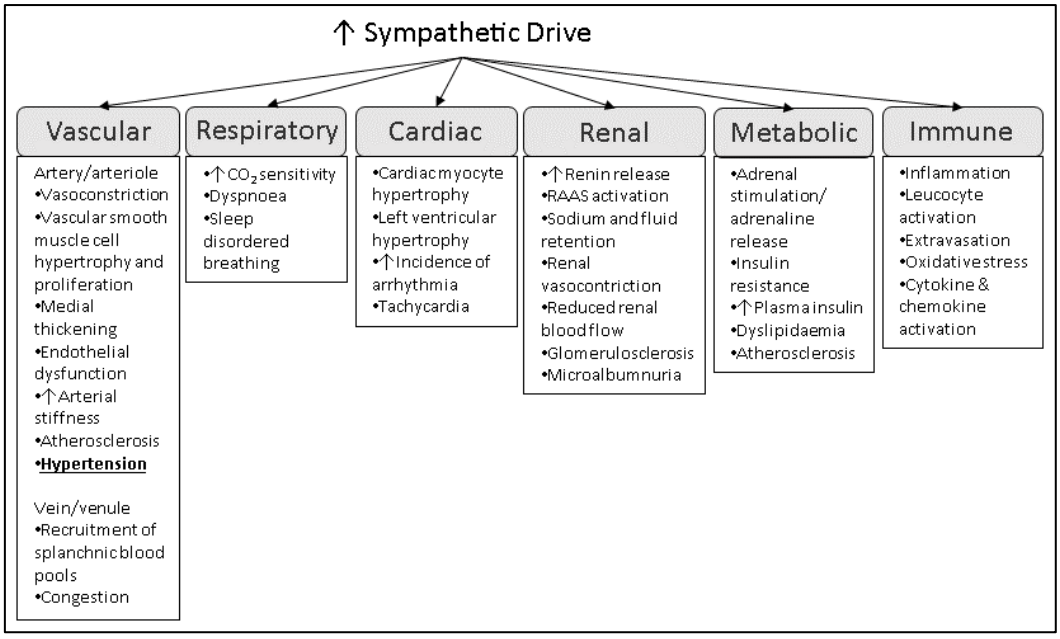


Figure 2-5. Consequences of raised sympathetic nerve tone
Adapted from Fisher and Paton, 2012 and Sobotka et al., 2011 (Sobotka, Mahfoud et al. 2011, Fisher and Paton 2012).

2.1.2.3 Causes of chronically elevated sympathetic tone

The concept of neurogenic hypertension develops the hypothesis that high blood pressure is driven by raised sympathetic nerve activity. This sympathoexcitation is caused by a variety of factors including rises in Ang II, inflammation, cerebral hypoperfusion and vascular dysfunction (see Figure 2-4). Alterations in other pathways with feedback to the vasomotor centre, including signals transmitted via the afferent renal nerves and alterations in baroreflex and peripheral chemoreflex sensitivity, along with metabolic dysfunction, have also been implicated in the aetiology of raised SNA and neurogenic hypertension (Mancia, Grassi et al. 1999, Grassi, Seravalle et al. 2010). It is estimated that at least 50% of cases of essential hypertension have an underlying neurogenic component (Esler 2010), and therefore understanding these mechanisms and developing techniques to modulate these pathways will hopefully provide novel treatment strategies for these individuals.

2.1.2.3.1 Impaired brain blood flow and the Cushing mechanism

I have already introduced the concept of the 'Selfish Brain Hypothesis', under which the brain will act to maintain cerebral perfusion and oxygenation, even at the detriment of raised systemic arterial pressure (see section 2.1.1.5). This is refined by Paton at al. as Cushing's mechanism (Paton, Dickinson et al. 2009), through which brainstem hypoperfusion activates the sympathoexcitatory networks to increase TPR and therefore increased systemic arterial pressure, but what is the evidence that cerebral hypoperfusion causes long term arterial hypertension?

Cerebral hypoperfusion can occur due to increase cerebral vascular resistance (CVR) (Paton, Dickinson et al. 2009). As early as 1948 Kety demonstrated that CVR rose in

accordance with arterial pressure in people with essential hypertension despite relatively normal levels of cerebral blood flow (CBF) (Kety, Hafkenschiel et al. 1948); whilst it could be hypothesised that increased CVR is due to increased arterial pressure, the converse could also be true and raised CVR could result in raised arterial pressure in order to maintain CBF. Post mortem studies by Dickinson and Thomson demonstrated that people with previous hypertension had stenotic vertebral and carotid arteries, and that hypertensives had increased vascular resistance in the arteries supplying the brainstem (Dickinson and Thomson 1960). Our group has demonstrated an increased prevalence of vertebral artery hypoplasia and incomplete Circle of Willis in patients with hypertension (Rodrigues, Hart et al. 2015), particularly those with resistant hypertension, and Hart et al. have shown that these vascular abnormalities are not only more prevalent in hypertensives, but associated with increased CVR and decreased CBF (Warnert, Rodrigues et al. 2016). Looking prospectively, CVR was elevated before, and predictive of, the onset of hypertension in humans (Warnert, Rodrigues et al. 2016).

Further insight has been obtained from an animal model for hypertension, the spontaneously hypertensive rat (SHR). Paton's group has shown that pre-hypertensive (i.e. young pups) SHR rats already have raised SNA, as well as vertebral artery remodelling, and increased brainstem vascular resistance compared to age- and sex-matched normotensive rats (Cates, Steed et al. 2011). Additionally, occlusion of both vertebral arteries in the young, pre-hypertensive SHR resulted in a greater increase in SNA compared with aged matched controls, suggesting that vascular remodelling, increased CVR and a raised responsiveness to brainstem hypoperfusion (a sensitised Cushing mechanism) are all present in these animals prior to development of fulminant hypertension (Cates, Steed et al. 2011).

So how does brainstem hypoperfusion cause an increase in SNA: how is the Cushing's mechanism activated? The site/s of the central sensor for the Cushing's mechanism remains to be confirmed, although the RVLM, and particularly the NTS, are likely candidates (Paton, Dickinson et al. 2009, Cates, Dickinson et al. 2012). SHR have a shift from oxidative to non-oxidative metabolism in the brainstem (Paton, Wang et al. 2008), and Paton et al. hypothesise that the brainstem in hypertensive humans may have a limited capacity for oxidative metabolism if it is already running borderline hypoxic due to increased CVR, and that the consequent shift to non-oxidative metabolism, with the production of non-oxidative metabolites including reactive oxygen species, may act locally to stimulate increased sympathetic outflow from the vasomotor centre (Peterson, Sharma et al. 2006, Paton, Dickinson et al. 2009). Increased CVR also blunts the central transmission of the baroreflex, so that there is limited opposition to any increase in arterial pressure (Paton, Dickinson et al. 2009).

2.1.2.3.2 A long-term role for the baroreflex in hypertension

There is evidence that arterial *baroreflex sensitivity* (BRS) is impaired in hypertension, dampening the reflex inhibition of sympathetic overdrive (Matsukawa, Gotoh et al. 1991, Pikkujamsa, Huikuri et al. 1998, Ding, Zhou et al. 2011). The baroreflex both declines in sensitivity and re-sets to operate over higher pressures in chronic hypertension, as illustrated by a progressive loss of baroreceptor buffering of mean arterial pressure and renal SNA in the older SHR (Judy and Farrell 1979). This may be

associated with increased arterial stiffness, hence reduced carotid distensibility, and/or central nervous system re-setting with decreased excitability of the brainstem reflex or decreased transduction (Paton, Dickinson et al. 2009).

Is impaired BRS a cause or consequence of hypertension? Sympathetic BRS has been shown to be impaired in patients with borderline hypertension (Matsukawa, Gotoh et al. 1991), and reduced BRS has been implicated in the pathogenesis of hypertension. Cardiac BRS is impaired in young, pre-hypertensive SHR (Minami, Imai et al. 1989). In humans, sympathetic vascular BRS is impaired in normotensive adolescents with a family history of hypertension (Yamada, Miyajima et al. 1988), and adolescents with white coat hypertension have reduced cardiac BRS which provides an early marker for the onset of hypertension (Honzikova and Fiser 2009). Reduced BRS is an independent indicator for all-cause mortality and of cardiovascular morbidity in hypertensive patients (and in other conditions of sympathetic over activity) (Johansson, Gao et al. 2007, Ormezzano, Cracowski et al. 2008).

In the classic Guytonian paradigm, the baroreflex resets in response to sustained changes in the level of arterial pressure and therefore has no role in the long-term regulation of blood pressure. More recently there is evidence to counter this view. Thrasher demonstrated that chronic unloading of the carotid baroreceptors (by ligation of the common carotid artery proximal to the sinus in a canine model) could produce arterial hypertension. Lohmeier et al. have investigated the effect of chronic baroreflex activation using electrical stimulation, which achieved significant reduction in MAP (Iliescu and Lohmeier 2009, Lohmeier and Iliescu 2015). Mechanisms for this effect include incomplete central resetting of the baroreflex, and suppression of the RAAS system due to decreased renal sympathetic nerve mediated renin release, although this requires elucidation (Lohmeier and Iliescu 2015). The central component of the baroreflex has been shown to reset; as described above, Ang II, acting centrally on the NTS depresses the baroreflex (cardiac and sympathoinhibitory pathways), potentiating hypertension (Tan, Killinger et al. 2007, Paton, Wang et al. 2008).

2.1.2.3.3 Visceral afferent hyperactivity

Koeners et al. have recently highlighted the importance of visceral afferent hyperactivity in the pathogenesis of hypertension (Koeners, Lewis et al. 2016). They consider the problem of a supply-demand mismatch resulting for impaired visceral autoregulation: under pathological conditions metabolically sensitive afferents that trigger increased SNA can become sensitised and hyper-reflexic, generating raised sympathetic tone (Koeners, Lewis et al. 2016). In the context of increased oxygen demand, if local autoregulatory mechanisms of vasodilation become saturated, then there is a failure of functional sympatholysis, and the vasoconstrictor action of SNA caused by afferent sensitisation may overcome the increased perfusion driven by local autoregulation. Administration of Ang II results in an increase in reactive oxygen species within skeletal muscle and disrupts the nitric oxide synthase dependent attenuation of sympathetic vasoconstriction (Zhao, Swanson et al. 2006).

There are several examples of visceral afferent hyperactivity, including sensitisation of the afferent renal nerves, carotid body chemohypersensitivity and sensitisation of the

adipose afferent reflex in hypertension (Sinski, Lewandowski et al. 2012, Xiong, Chen et al. 2012, Koeners, Lewis et al. 2016). Abnormal afferent nerve activity from skeletal muscle has also been observed. Skeletal muscle afferents comprise stretch-activated mechanoreceptors and metabolically sensitive metaboreceptors. In humans, the vasoconstrictor effect of SNA is usually blunted in exercising muscle to improve local blood flow, however, this functional sympatholysis is impaired in patients with hypertension (Vongpatanasin, Wang et al. 2011). In patients with heart failure, passive leg movement resulted in increased norepinephrine spillover and arterial pressure, but reduced femoral blood flow, indicating abnormal muscle afferent feedback (Ives, Amann et al. 2016). The exercise pressor response (an increase in arterial pressure, heart rate, ventilation, stroke volume and SNA, and the redistribution of blood to exercising muscle) is exaggerated in hypertension (Li and Xing 2012), and an increased pressor response to exercise amongst normotensive humans is associated with the development of hypertension (Berger, Grossman et al. 2015).

It is well established that obesity can drive sympathoexcitation (DiBona 2013); rabbits develop an increase in weight, mean arterial pressure, heart rate, renal SNA, and insulin and leptin levels within one week of starting a high-fat diet (Armitage, Burke et al. 2012), and rats fed a high-fat diet have an increase in adipose tissue and leptin levels which precede a rise in SNA (Muntzel, Al-Naimi et al. 2012). Increased leptin and insulin levels have therefore been implicated in the development of hypertension (Armitage, Burke et al. 2012, DiBona 2013). Stimulation of adipose tissue using capsaicin results in an increase in arterial pressure and SNA via the adipose afferent reflex (Xiong, Chen et al. 2012). This reflex is sensitised in obese animals and can be blocked via injection of lignocaine into the PVN (paraventricular nucleus), demonstrating tonicity (Xiong, Chen et al. 2012).

Visceral afferent hyperactivity involving the kidneys and carotid bodies will now be discussed in more detail.

2.1.2.3.4 Peripheral chemoreceptor reflexes in hypertension

In human and preclinical models of hypertension peripheral chemoreceptor reflexes are modulated making them pro-sympathoexcitatory.

The *peripheral chemoreflex* is predominantly mediated via the highly-perfused carotid bodies (Paton, Sobotka et al. 2013). Activation of the chemoreflex by hypoxia, hypercapnia or increase hydrogen ion concentration, causes sympathoexcitation via action on the NTS, RVLM and PVN (Koeners, Lewis et al. 2016). Schultz's group have demonstrated that hypoperfusion of the carotid body in a rabbit model of chronic heart failure increases chemoreflex sensitivity, and primarily attribute the consequent maladaptive increase in sympathetic tone to carotid body afferent drive (Ding, Li et al. 2011, Schultz, Marcus et al. 2013). In human normotensive controls, the MSNA response to hypoxia is augmented by prior exposure to repeated hypoxic apnoeas (Cutler, Swift et al. 2004); this increased chemosensitivity helps to explain the pathological relationship seen between the intermittent hypoxias of obstructive sleep apnoea and hypertension (Weiss, Liu et al. 2007). Relative to normotensive control animals, SHR exhibit elevated sympathoexcitatory responses to peripheral chemoreceptor stimulation, and the

peripheral chemoreceptors exert a tonic excitatory influence on sympathetic activity (Abdala, McBryde et al. 2012). Raised tonic chemoreceptor activity has also been demonstrated in hypertensive humans; these patients had elevated baseline MSNA which was reduced in response to exposure to hyperoxia in contrast to normotensive controls who had normal baseline MSNA with no change in response to a hyperoxic stimulus (Sinski, Lewandowski et al. 2012). Denervation of the carotid body in both pre-hypertensive SHR and rats with established hypertension results in a reduction in BP and sympathetic vasomotor tone, suggesting an intrinsic role for chemosensitivity in both the development and maintenance of hypertension (Abdala, McBryde et al. 2012, McBryde, Abdala et al. 2013). Increased chemosensitivity is also observed in the early stages of human hypertension, supporting this causal relationship (Trzebski, Tafil et al. 1982, Somers, Mark et al. 1988).

A joint study between our team in Bristol, led by Prof. Paton, and the team in Gdansk, Poland, led by Prof. Narkiewicz has demonstrated the safety and feasibility of unilateral carotid body resection in patients with hypertension (Narkiewicz, Ratcliffe et al. 2016). The study showed a reduction in ambulatory BP and MSNA in 8 out of 15 participants three months after surgery. This effect was sustained out to 6 months, before returning towards baseline levels, potentially reflecting adaptation from the remaining carotid body.

Evidence is now in place for a novel pharmacological agent to inhibit chemoreceptor activation in hypertension. Pijacka et al. have demonstrated the upregulation of the purinergic receptor P2X3 in chemoreceptive petrosal sensory neurons in rats with hypertension (Pijacka, Moraes et al. 2016). Antagonism of P2X3 receptors inhibited both the tonic and hyperreflexic chemoreceptor activity seen in the hypertensive (but not normotensive) rats, and furthermore, was shown to reduce BP and SNA in the hypertensive animals. The authors also demonstrated P2X3 receptor expression in human carotid bodies and hyperactivity of carotid bodies in individuals with hypertension, thus P2X3 receptor antagonists may represent an exciting novel pharmacological approach for the treatment of hypertension (Paton, Sobotka et al. 2013, Pijacka, Moraes et al. 2016).

2.1.2.3.5 The role of the renal afferent nerves in sympathoexcitation

Renal afferent nerves project to, and terminate in, the dorsal horn of the thoracic spinal cord. Signals are then relayed to the brain and trigger activation of brainstem and hypothalamic neurones that control sympathetic activity (Guyenet 2006). Renal afferents are both mechanically and chemically sensitive. Mechanoreceptors, located primarily in the renal pelvis, are sensitive to increases in intrarenal pressure caused by renal compression, renal vein occlusion or increased arterial perfusion pressure (Nijima 1971, Winternitz, Katholi et al. 1980, Johns, Kopp et al. 2011, Koeners, Lewis et al. 2016). Renal chemoreceptors respond to hypoxia and changes in the ionic composition of the renal pelvis (Winternitz, Katholi et al. 1980).

Figure 2-6 illustrates factors that can drive renal afferent nerve activity; these include ischaemia, adenosine hypoxia, inflammation and chronic kidney disease (CKD) (Converse, Jacobsen et al. 1992, Campese and Kogosov 1995, Hausberg, Kosch et al.

2002, Sobotka, Mahfoud et al. 2011). This response to renal injury has been demonstrated in a rat model using an injection of phenol (causing local haemorrhage, congestion and necrosis (Ye, Gamburd et al. 1998)) into the kidney, which was shown to cause an increase in BP and SNA; subsequent renal denervation reverses this effect and normalises BP and SNA, confirming that it is the afferent nerve signal, rather than hormonal changes, that have mediated the response to phenol (Campese 1997, Ye, Zhong et al. 2002). Bilateral dorsal rhizotomy, disrupting the afferent renal nerves, prevents the development of hypertension in rats with a 5/6 model of CKD (Campese and Kogosov 1995).

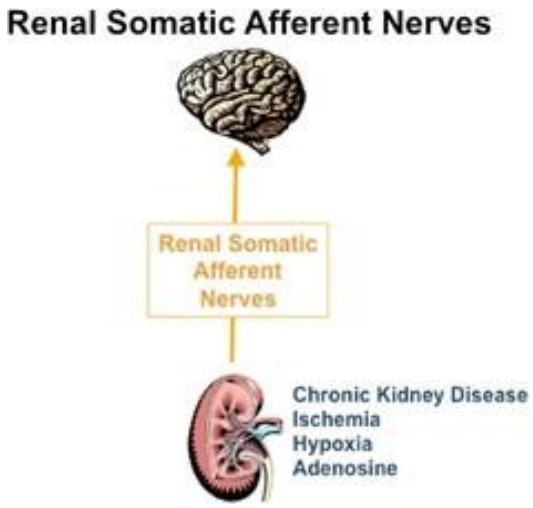


Figure 2-6. Renal sensory nerve activity, a source of reflexly elevated sympathetic tone. (Sobotka, Mahfoud et al. 2011)

Patients with CKD on haemodialysis have MSNA levels 2.5 times that of healthy controls (Converse, Jacobsen et al. 1992). Following renal transplantation, patients whose diseased kidneys are left in situ continue to have raised SNA despite normalisation of their renal function, that it is the afferent signals from the disease kidney (e.g. fibro-proliferative scarring, local ischaemic or the release of mediators such as adenosine), rather that the impairment in renal function or uraemic milieu, that drives sympathoexcitation (Hausberg, Kosch et al. 2002). In contrast, SNA in patients who have undergone bilateral nephrectomy at the time of transplantation returns to normal, once again emphasising the importance of the afferent signals from the diseased kidney in driving elevated SNA, although renin release due to renal hypoperfusion, and activation of the RAAS could also provide an 'afferent' signal through central action of Ang II (Hausberg, Kosch et al. 2002).

Intra-renal adenosine has both systemic effects on blood pressure (Katholi, Hageman et al. 1983), and local autoregulatory effects (Wierema, Houben et al. 2005). Adenosine is released in the kidney in response to hypoxia (Miller, Thomas et al. 1978), and has been shown to activate the afferent renal nerves located in the renal pelvis, primarily through action on A1 receptors, to cause a systemic rise in arterial pressure (Katholi 1983, Katholi, Hageman et al. 1983, Ma, Liu et al. 2004). In a one kidney, one-clip rat model of

hypertension, urinary adenosine concentration was lowered by infusion of adenosine deaminase into the renal artery. When urinary adenosine levels fell, sympathetic nerve activity and hypertension were blunted; this effect was abolished by RDN (Katholi, McCann et al. 1985). In chronically instrumented, uni-nephrectomised sodium-replete conscious dogs, an increase in systemic arterial BP seen in response to intra-renal arterial adenosine infusion (0.6-3 mcg/kg/min)was abolished by renal artery denervation due to the interruption of the renal afferent nerve fibres (Katholi, Whitlow et al. 1984).

When considering local haemodynamic effects, animal models indicate that adenosine acts via high affinity A1 receptors which trigger vasoconstriction and activate the tubuloglomerular feedback system, and lower affinity A2 receptors which cause vasodilatation and inhibition of tubular sodium reabsorption (Biaggioni 1992). In vitro studies using a blood-perfused rat juxtamedullary nephron preparation, demonstrated afferent and efferent renal arteriolar A1 receptor mediated vasoconstriction in response to superfusion of 1, 10 and 100 micromol/l adenosine, which was partly buffered by A2a receptor mediated vasodilatation (Nishiyama, Inscho et al. 2001). In man, intra-renal arterial adenosine administration has been shown to cause both renal vasoconstriction (e.g. in response to 1ml boluses of 10^{-5}-1 mg/ml adenosine (Marraccini, Fedele et al. 1996)) and vasodilatation (at concentrations in the range of 1-10 mcg/kg/min (Smits, de Leeuw et al. 1991, Wierema, Houben et al. 2005)).

Bradykinin also causes a reduction in RBF and a reflex increase in systemic mean arterial pressure (MAP) when infused into the renal artery in rats, an effect that is, abolished by RDN (Smits and Brody 1984). Foss et al. have developed a novel method for selective disrupting afferent renal nerve signals in a rat model, using peri-axonal capsaicin (an agonist of the transient receptor potential (TRP) V1 receptor) (Foss, Wainford et al. 2015). In these animals, blockade of the afferent renal nerves abolished the rise in MAP seen following intrarenal bradykinin infusion, and blunted the development of deoxycorticosterone acetate-salt hypertension[2] to the same extent as surgical RDN, indicating the anti-hypertensive effect of RDN in this model was primarily due to disruption of the afferent, rather than efferent renal nerves (Foss, Wainford et al. 2015).

Afferent neurones also project to the contra-lateral kidney generating a tonically active, inhibitory reno-renal reflex (Johns, Kopp et al. 2011, Kopp 2015); as evidenced by the abolition of reflex responses to contralateral afferent nerve stimulation or ureteric occlusion by spinal cord transection (Calaresu, Kim et al. 1978, Protasoni, Golin et al. 1996). Spontaneously hypertensive rats have an impaired reno-renal reflex (defect at the level of the renal chemo- and mechano-sensory receptors) which may contribute to their increased level of efferent renal SNA (Kopp, Smith et al. 1987, Kopp, Cicha et al. 2011, DiBona 2013).

There is increasing evidence for a central role for renal afferent nerves in the pathogenesis of hypertension; even a small degree of renal injury/hypoxia (not effecting renal function) can be sufficient to increase blood pressure (Koeners, Lewis et al. 2016). Stimulation of the afferent renal nerves in hypertension due to increase arterial

[2] DCOA-salt hypertension model: similar to mineralocorticoid excess in humans, results in volume overload, very high levels of DOC and salt intake required, progresses rapidly with severe hypertension and hypertrophy, therefore not suitable for long-term studies. Dornas, W. C. and M. E. Silva (2011). "Animal models for the study of arterial hypertension." J Biosci **36**(4): 731-737.

pressure, microvascular damage, Ang II mediated hypoperfusion, hypoxia and inflammation, may act to drive up blood pressure through a reflex increase in SNA, creating an adverse positive feedback loop (Koeners, Lewis et al. 2016). Disruption of these afferent signals therefore represents yet another therapeutic target for the treatment of hypertension and will be explored in this study.

2.1.2.3.6 Sympathoexcitation via angiotensin II

Angiotensin II increases blood pressure through a range of mechanisms, including increased renal sodium and water reabsorption and peripheral vasoconstriction. However, Ang II also acts centrally to increase SNA, which feeds into a positive feedback loop by stimulating renin release and further Ang II production through activation of the RAAS.

In the SHR levels of Ang II are increased in several areas of the brain, including the RVLM and NTS, compared to normotensive controls, and SHR also have a heightened pressor response to microinjection of Ang II into these areas (Veerasingham and Raizada 2003). Furthermore, intracerebroventricular infusion of the AT1R blocker losartan blocked the development of salt-sensitive hypertension in SHR (Huang and Leenen 1996, Veerasingham and Raizada 2003), and bilateral microinjection of peptide AT1R antagonists into the RVLM reduced both SNA and blood pressure in the SHR model of hypertension (Ito and Sved 1996, Veerasingham and Raizada 2003). Ang II also acts on the NTS to decrease baroreflex gain which may also contribute to the development of hypertension (Kasparov and Paton 1999, Paton and Kasparov 1999, Paton, Waki et al. 2003, Veerasingham and Raizada 2003, Paton, Wang et al. 2008). The situation may be even more complex; McBryde et al. have shown that high central doses of Ang II have a sympathoinhibitory effect due to baroreflex activation, but that a lower dose, associated with more gradual onset hypertension, can be sympathoexcitatory, showing a synergistic hypertensive action with increased salt intake (due to Ang II mediated potentiation of the osmoreceptors in the circumventricular organs) (McBryde, Guild et al. 2007),

Ang II mediated hypertension causes central inflammation, with elevated levels of TNFα (tumour necrosis factor-alpha), NF-κβ and reactive oxygen species (Fisher and Paton 2012), which may have a key role to play in the development of neurogenic hypertension. For example, the enzyme nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase, which plays a central role in the generation of reactive oxygen species, can be upregulated via activation of the AT1R (Gao, Wang et al. 2005, Fisher and Paton 2012), and specifically infusion of Ang II into the RVLM increases NADPH expression, reactive oxygen species levels and renal SNA (Gao, Wang et al. 2005). Ang II also stimulates T cell activation and the production of pro-inflammatory cytokines (Guzik, Hoch et al. 2007). This central inflammation can result in increased SNA and systemic hypertension (Fisher and Paton 2012).

2.1.2.3.7 Inflammation and raised sympathetic nerve activity

As introduced above, systemic and central inflammation are increasingly seen to play an important role in the development of hypertension. Levels of systemic inflammatory markers such as such as TNFα, interleukin-6, C-reactive protein (CRP) and adhesion molecules are increased in hypertension and may have a pro-hypertensive role (Fisher and Paton 2012). Disruption of T cell function, the use of anti-TNFα therapy (etanercept) and blockade of the pro-inflammatory NF-κβ signalling pathway are protective against the development of Ang II mediated hypertension (Muller, Dechend et al. 2000, Guzik, Hoch et al. 2007, Vinh, Chen et al. 2010). Systemic inflammation can upregulate microglia, pro-inflammatory cytokines and reactive oxygen species within the rostral ventrolateral medulla, with an associated rise in blood pressure and increased sympathetic vasomotor tone (see section 2.1.1.5) (Wu, Chan et al. 2012). Furthermore, Marvar et al. demonstrated that blockade of central Ang II action using an electrolytic lesion of the anteroventral third ventricle (abolishes virtually all of the central actions of Ang II), reduced peripheral T-cell activation, and also provided evidence of a feed-forward loop whereby high blood pressure in itself can cause T-cell activation (so called baro-trauma), which in turn acts to worsen hypertension (Marvar, Thabet et al. 2010).

Waki et al. demonstrated that inducing inflammation in the brainstem of normotensive rats, specifically with elevated levels of junctional adhesion molecule-1 (JAM-1), a major chemoattractant, in the NTS, resulted in the development of raised arterial pressure (Waki, Liu et al. 2007). Additionally, there was upregulation of JAM-1 in the NTS of SHR with evidence of invasion of leukocytes which predated the onset of hypertension (Waki, Liu et al. 2007). An association has since been found between peripheral levels of JAM-1 and hypertension in a Hong Kong population, associated with specific single nucleotide polymorphisms (SNPs) (Ong, Leung et al. 2009). This has raised the question as to whether an inflammatory process, in which JAM-1 is upregulated centrally, may drive hypertension through excessive SNA originating from a relatively under-perfused brainstem (Fisher and Paton 2012). Central NF-κβ activation by Ang II in the paraventricular nucleus of the hypothalamus has also been shown to increase sympathetic nerve activity (Kang, Ma et al. 2009).

Additional data come from further animal studies; Wu et al. reported that systemic inflammation promoted sustained hypertension, and induced not only rises in the plasma level of CRP, TNFα and interleukin-1β (IL-1β), but also resulted in activation of microglia and increased levels of IL-1β, IL-6, TNFα reactive oxidative species in the RVLM (Wu, Chan et al. 2012). Importantly, both the pressor response and the central inflammatory changes were blunted by intracisternal infusion of either a cycloxygenase-2 (COX-2) inhibitor, minocycline (and anti-inflammatory antibiotic which inhibits microglial activation), or pentoxifylline (a cytokine synthesis inhibitor), provided that these agents were administered centrally (Wu, Chan et al. 2012). Likewise, the peripheral administration of TNFα and direct injection of prostaglandin-E2 into the paraventricular nucleus or RVLM, both resulted in an increase in arterial pressure and SNA; the pressor effect of the prostaglain-E2 administration was blocked by central COX-2 inhibition (Zhang, Wei et al. 2003). In wildtype mice, stress-related hypertension is associated with T cell activation (and augmented by Ang II), however, in T-cell deficient RAG knockout mice repeated stress did not cause hypertension, furthermore, the increase in BP in response to stress was restored by adoptive transfer of T cells into the deficient strain (Marvar, Vinh et al. 2012). In humans, the angiotensin receptor blocker valsartan has been shown to reduce both arterial pressure and levels of the pro-

inflammatory cytokines TNFα and IL-6. The role of inflammation, particularly central inflammation, in driving high blood pressure may therefore represent a novel therapeutic target for the clinical management of hypertension.

2.1.2.3.8 Hypertensive Hypotheses

The pathogenesis of human hypertension is a complex process, with a chronic imbalance in the homeostatic reflexes which have evolved to protect us from insults such as dehydration, haemorrhage and hypoxia. Guyton placed the kidney at the centre of blood pressure control, and primarily attributed hypertension to an alteration in salt/volume loading or renal sodium handling, but as discussed, the situation is far more complex (Guyton and Hall 1996). Increased activity in the RAAS, with emphasis on both the central and peripheral actions of Ang II, is clearly partly to blame (Guyenet 2006). Whilst Ang II can act to drive sympathoexcitation, the concept of neurogenic hypertension with associated sympathetic overdrive, and the importance of afferent feedback from the kidneys, carotid bodies, adipose tissue and skeletal muscle, as the primary driver in the development and maintenance of high BP, continues to gain momentum (Fisher and Paton 2012, Koeners, Lewis et al. 2016). None of these hypotheses stand alone, and the central reflexes which aim to preserve cerebral blood flow and the pro-inflammatory milieu seen in patients with (and even prior to the development of) hypertension, provide further therapeutic targets for the treatment of this condition (Cesari, Penninx et al. 2003, Paton, Dickinson et al. 2009, Zubcevic, Waki et al. 2011). It is likely that the predominant mechanism driving hypertension will vary between subjects, and the ability to establish the autonomic profile of a patient would enable physicians to tailor their therapeutic approach to the individual. Given the prevalence of treatment resistant hypertension, and the rapidly evolving field of interventional therapy in hypertension, a firm understanding of the pathology underlying both treatment failure and potential targets for future success is key, and I will now go on to explore the clinical challenge of hypertension resistant to existing pharmacological therapy.

2.2 Resistant Hypertension: The clinical challenge

Hypertension presents a significant challenge for health care providers, with the world-wide prevalence predicted to rise to 1.56 billion by 2025 (Kearney, Whelton et al. 2005). In England 31% of men and 26% of women have been diagnosed with hypertension (Townsend, Bhatnagar et al. 2017). In those receiving treatment, 6% have high blood pressure (BP) that remains uncontrolled, furthermore, in 16% of men and 11% of women, BP remains untreated (Townsend, Bhatnagar et al. 2017). There are multiple causes for resistant hypertension, and differentiating between patients with true drug resistance, as opposed to those with pseudo-resistance due to factors such as poor medication adherence, drug intolerance or secondary hypertension, will help to guide therapeutic strategies.

2.2.1 Definition and diagnosis of hypertension

Hypertension is defined as an office (clinic) systolic blood pressure (SBP) of ≥140 mmHg and/or an office diastolic blood pressure (DBP) of ≥90 mmHg (Mancia, Fagard et al. 2013). Blood pressure and its relationship to cardiovascular risk lie on a continuum, but for practical purposes different levels of hypertension have been defined. The definitions differ slightly between the National Institute of Health and Care Excellence (NICE) in the UK, and the joint European Society of Cardiology/ European Society of Hypertension (ESC/ESH) guidelines (see Table 2-1 and Table 2-2, respectively). Importantly, the 2011 NICE guidelines recommend ambulatory blood pressure monitoring (ABPM) in patients with a clinic BP of ≥140/90 mmHg to confirm hypertension and address the issue of white-coat hypertension. If ABPM is not possible or not tolerated, then home BP monitoring (HBPM) is advised with similar numerical cut-off points between stages of hypertension. It is important to remember that these definitions do not take into account the aetiology of the raised BP, which may differ between individuals.

These definitions are based on evidence targeting blood pressure control to <140/90 mmHg (National Clinical Guideline 2011), however more recent data from the SPRINT trial has shown a reduction in major cardiovascular events and death following intensive (target SBP <120 mmHg) as opposed to standard (SBP< 140 mmHg) blood pressure control among patients at high risk for cardiovascular events but without diabetes (Wright, Williamson et al. 2015). In light of these data it may be that future guidelines advocate a lower blood pressure threshold and treatment target.

Stage	Clinic BP	ABPM daytime average	Recommendation
Normotensive	<140/90	<135/85	Offer BP check at least every 5 years
Normotensive (White-coat)	≥140/90	<135/85	Offer BP check at least every 5 years

Stage 1	≥140/90	≥135/85	Offer lifestyle interventions If target organ damage or 10-year cardiovascular risk >20% offer antihypertensive treatment
Stage 2	≥160/100	≥150/95	Offer antihypertensive treatment
Severe	Systolic>180 or Diastolic>110		Consider starting antihypertensive drug treatment immediately. If accelerated hypertension or suspected phaeochromocytoma same day specialist referral.

Table 2-1. The Stages of Hypertension. (National Clinical Guideline 2011)
Blood pressure in mmHg. If younger than 40 years, consider specialist referral.

Category	**Systolic**		**Diastolic**
Optimal	<120	And	<80
Normal	120-129	and/or	80-84
High normal	130-139	and/or	85-89
Grade 1 hypertension	140-159	and/or	90-99
Grade 2 hypertension	160-179	and/or	100-109
Grade 3 hypertension	≥180	and/or	≥110
Isolated systolic hypertension	≥140	And	<90

Table 2-2. Definitions and classification of office blood pressure (Mancia, Fagard et al. 2013).
Blood pressure in mmHg.

Ambulatory BP monitoring goes a long way to address the diagnosis of white-coat hypertension, although some patients with clinically significant hypertension may still be missed, including those with masked hypertension (office BP <140/90 mmHg, but ABPM/HBPM >135/85 mmHg), a non-dipping blood pressure profile, or a pronounced early morning surge despite a normal range mean BP on ABPM; these individuals remain at increased cardiovascular risk compared to the normotensive population (Pickering, Eguchi et al. 2007, Pierdomenico, Pierdomenico et al. 2017).

Blood pressure is the product of blood flow (with total blood flow equalling cardiac output) and peripheral vascular resistance. In patients with established hypertension the primary abnormality is an increase in total peripheral resistance rather than an increase in cardiac output (Lund-Johansen 1983). Patients with increased arterial stiffness, who are frequently more elderly, may have isolated systolic hypertension (ISH, BP ≥140/<90 mmHg). ISH is not a benign phenomenon and antihypertensive treatment reduces mortality and morbidity, however the aggressive BP control indicated by the SPRINT trial

may not translate to all groups with hypertension (Bavishi, Goel et al. 2016). Caution is required in elderly patients, with results from the Predictive Values of Blood Pressure and Arterial Stiffness in Institutionalized Very Aged Population (PARTAGE) study suggesting that tight SBP control in frail elderly patients may have a negative effect on mortality and cognition (Benetos, Labat et al. 2015, Mossello, Pieraccioli et al. 2015, Kulenthiran, Ewen et al. 2017). Interestingly, the PARAMETER (Prospective Comparison of ARNI with ARB Measuring Arterial Stiffness in the Elderly) study, which looked at the effect of LCZ696 (sacubitril/valsartan; a combined angiotensin receptor neprilysin inhibitor (ARNI) and angiotensin receptor blocker (ARB)) on central aortic haemodynamics and arterial stiffness in comparison to the ARB olmesartan, showed that the combined drug was more effect at reducing central BP and pulse pressure than the ARB alone, without any increase in adverse events (Williams, Cockcroft et al. 2015, Kulenthiran, Ewen et al. 2017). The results of these recent studies emphasise the importance of individualised patient care in hypertension. A raised BP may give a diagnosis of hypertension but does not reflect the cause of the condition which may be wide ranging (Section 2.1 and Box 2.1).

2.2.1.1 Definition of resistant hypertension

Failure to achieve blood pressure control despite treatment is known as resistant hypertension, however, this broad term covers both drug resistant hypertension, and pseudo-resistant hypertension which can be attributed to a range of pathological and practical problems. Drug resistant hypertension is more specifically defined as the failure to achieve an office BP of <140/90 mmHg despite compliance with ≥3 anti-hypertensive medications including a diuretic or, by some parties, as any BP (controlled or uncontrolled) on 4 or more agents (Calhoun, Jones et al. 2008). The challenges presented by pseudo-resistance are discussed below.

The term refractory hypertension is increasingly being used to describe the failure to control BP with use of ≥5 antihypertensive medications, with the most rigorous definition requiring the use of ≥5 agents, including a long-acting thiazide or thiazide-like diuretic and an aldosterone antagonist, such as spironolactone (Cai and Calhoun 2017).

2.2.2 Pseudo-resistant hypertension

White-coat effect (a discrepancy of more than 20/10 mmHg between clinic and average daytime ABPM) is only one explanation for pseudo-resistance in patients treated for hypertension. Causes of resistant hypertension, including pseudo-resistant and secondary hypertension are summarised in Box 2.1. Patients presenting with resistant hypertension or young onset hypertension (age <40 years), warrant further investigation for secondary causes (National Clinical Guideline 2011).

2.2.2.1 Secondary hypertension

Patients diagnosed with resistant hypertension are more likely to have previously unidentified secondary causes for their high BP (Limonta, Valandro Ldos et al. 2012). Common contributors include high salt intake, excess alcohol intake, obesity, obstructive

sleep apnoea (OSA), chronic kidney disease, renal artery stenosis and hyperaldosteronism, with other secondary causes being hyper- or hypothyroidism, Cushing's Syndrome, phaeochromocytoma, hyperparathyroidism and aortic coarctation (De Nicola, Borrelli et al. 2011, Oliveras and Schmieder 2013). Co-prescribing of hypertensive medications including non-steroidal anti-inflammatory drugs, decongestants, oral contraceptives and corticosteroids (see Box 2.1), can also make hypertension very difficult to control (Oliveras and Schmieder 2013). In a small study of 125 patients, OSA (apnea-hypopnea index (AHI) >15 events per hour) was the most common condition associated with resistant hypertension (64.0%), followed by primary aldosteronism (5.6%), renal artery stenosis (RAS, 2.4%), renal parenchymal disease (1.6%), oral contraceptives (1.6%), and thyroid disorders (0.8%) (Pedrosa et al. 2011). In 34.4% of these patients (43/125), no secondary cause of hypertension was identified giving a diagnosis of primary, and true resistant, hypertension (Pedrosa, Drager et al. 2011). In another study 16/83 (19%) of patients with apparent treatment resistant hypertension where found to have secondary causes for their high BP following investigation at a specialist hypertension clinic (seven had hyperaldosteronism (including two adrenal adenomas), three with RAS, three with CKD and three with OSA) (Heimark, Eskas et al. 2016).

Subclinical hyperaldosteronism and OSA have previously been particularly under-diagnosed (Logan, Perlikowski et al. 2001, Clark, Ahmed et al. 2012). Pimenta et al. studied 97 patients with resistant hypertension using polysomnography and plasma renin and urinary aldosterone levels, 77.3% had OSA and 28.9% had hyperaldosteronism (Pimenta, Stowasser et al. 2013). Lloberes et al. have published similar findings, with a prevalence of severe OSA (AHI >30) of 70% in those with resistant hypertension, and report that excessive daytime somnolence as assessed using the Epworth Sleepiness Scale could be a marker of a pathological mechanism linking OSA and hypertension (Lloberes, Lozano et al. 2010). A randomised controlled trial examining the use of continuous positive airway pressure (CPAP) in the treatment of those with OSA and resistant hypertension demonstrated a significant reduction in BP and an increase in the number of patients exhibiting a nocturnal dipping BP profile (Lozano, Tovar et al. 2010). OSA and HTN are both characterised by sympathoexcitation (Kario 2009). Treatment of OSA with CPAP has been shown to reduce muscle sympathetic nerve activity (MSNA) (Narkiewicz, Kato et al. 1999, Henderson, Fatouleh et al. 2016), providing a potential mechanism for the antihypertensive effect of CPAP in resistant hypertension.

Causes of resistant hypertension

- Pseudo-resistant hypertension
  - White coat hypertension
  - Non-adherence to medication
  - Physician inertia in optimising antihypertensive regimen
  - Poor BP measurement technique
- Secondary causes of hypertension, such as:
  - Vascular
    - Renal artery stenosis
    - Aortic coarctation
  - Renal parenchymal disease
  - Endocrine
    - Phaeochromocytoma
    - Primary hyperaldosteronism (Conn's syndrome)
    - Hypercortisolism (Cushing's syndrome)
    - Hyperparathyroidism
    - Hyper- and hypothyroidism
  - Obstructive sleep apnoea syndrome (OSAS)
  - Intracranial tumour, cerebral vasculitis
- Volume overload
  - Progressive renal insufficiency
  - High sodium intake
  - Inadequate diuretic therapy
- Drug induced hypertension
  - Non-steroidal anti-inflammatory drugs (NSAIDs)
  - Cocaine, amphetamines, other illicit drugs
  - Sympathomimetic agents
  - Oral contraceptive hormones
  - Ciclosporin, tacrolimus
  - Erythropoietin
  - Corticosteroids
  - Liquorice
  - Herbal compounds (ephedra, ma huang)
- Associated lifestyle conditions
  - Weight gain, obesity
  - Excessive alcohol intake

Box 2.1. Causes of resistant hypertension.
Adapted from Fagard 2012 and Calhoun et al. 2008. (Calhoun, Jones et al. 2008, Fagard 2012)

There has been ongoing debate about the prevalence of primary hyperaldosteronism as a secondary cause for hypertension. Primary hyperaldosteronism was identified in 7% of hypertensive patients in a German epidemiological study (Hannemann, Bidlingmaier et al. 2012), and in a separate cohort, 11.3% of patients with resistant hypertension were diagnosed with primary aldosteronism (Douma, Petidis et al. 2008). Notably, hypokalaemia was only seen in 45.6% of this latter group, and therefore may represent

a poor screening index for the condition. The authors are also critical of the aldosterone:renin ratio to screen for hyperaldosteronism, which had a sensitivity of only 53.8% in their cohort, and recommend suppression testing with either salt loading (2 litres of saline over 4 hours, sensitivity 97.3%, specificity 80.1%) or fludrocortisone dosing for 4 days (0.1mg every 6 hours, used as the gold standard in this study), however these tests are clearly more invasive and burdensome for the patients (Douma, Petidis et al. 2008). Investigating along similar lines, Martins et al report that 8% of patients with resistant hypertension studied had subclinical hypercortisolism; particularly older patients, those with diabetes and those with non-dipping nocturnal BP (Martins, Conceicao et al. 2012). Unfortunately, it can be difficult to screen for subclinical hyperaldosteronism routinely, due to the need to discontinue antihypertensive medications which may give false positive results, particularly beta blockers, in individuals with elevated BP (Schmiemann, Gebhardt et al. 2012), and a more pragmatic approach of introducing an aldosterone antagonistic such as spironolactone may be sensible in patients without electrolyte abnormalities or adrenal abnormalities on imaging.

2.2.2.2 Inadequate pharmacotherapy

Concordance with medication is an established problem, with two retrospective cohort studies reporting a medication discontinuation rate of around 40% (Mazzaglia, Mantovani et al. 2005, Van Wijk, Klungel et al. 2005). Low adherence was reported by 8.1% of those with apparent drug resistant hypertension in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) trial when questioned directly using a medication adherence scale (Irvin, Shimbo et al. 2012). Jung et al. investigated 375 patients with uncontrolled hypertension using toxicological urinalysis, after screening for white-coat hypertension, secondary hypertension and optimisation of antihypertensive therapy (including an increase to four drug therapy in 17 patients), 76 patients remained; of these, 53% were non-adherent (Jung, Gechter et al. 2013). A population-based study from Israel (172,432 patients) reported uncontrolled BP in 35.9% of patients. In the majority of cases these patients were undertreated, with 21% receiving less than maximal dosages of prescribed medications, 9% not prescribed a diuretic, 48% dispensed <3 antihypertensive medications, and most worryingly, 20% having not been dispensed/not purchased any their prescribed blood pressure medication during a thirty day period (Weitzman, Chodick et al. 2014). Once these factors were excluded, only 2.2% of the patients in the study were defined as having true resistant hypertension.

A recent systematic review looking at the reasons for poor adherence with antihypertensive medications identified three consistent factors; higher financial cost to the patient for medication, side-effects causing discomfort (such as dry mouth, itching, tiredness, dizziness or sexual dysfunction) and a poor patient-provider relationship (van der Laan, Elders et al. 2017). The other factors associated with poor adherence are summarised in Box 2.2 (van der Laan, Elders et al. 2017). One strategy to target this poor adherence is the use of combination tablets, and there is increasing evidence to show that these medications can improve compliance and BP control (Egan, Bandyopadhyay et al. 2012). Further research is required to develop novel pharmacological agents with more tolerable side-effect profiles in order to improve quality of life for people with hypertension.

Patients with multiple drug intolerance also present a significant challenge for clinicians; in a condition which is largely asymptomatic, uncomfortable drug side effects and potentially expensive and onerous polypharmacy, can present a significant physical and financial burden for patients, and must be balanced against a far less tangible, statistical, cardiovascular disease risk. A lack of engagement with, or knowledge of, the current guidelines and evidence base for prescribing in hypertension amongst some medical professionals, or limited time and resources for regular patient contact to enable education, BP monitoring and drug titration, can also account for a failure in the optimisation of drug regimens. For example, beta blockers were previously widely used as a first line treatment for hypertension, but have now been shown to be significantly less effective than other agents, and even simple factors such as poor BP measurement technique (cuff too small, patient not sat quietly for long enough) can lead to an inaccurate diagnosis (National Clinical Guideline 2011).

Factors associated with non-adherence to antihypertensive medications

- Clinical factors
  - ***Side-effects causing discomfort***
  - High body mass index
  - Number of co-morbidities
  - Having diabetes, depression, history of cardiovascular disease
  - Duration of hypertension

- Social and demographic factors
  - Male gender
  - Younger age
  - Racial/ethnic minority status
  - Marital status
  - Employment
  - Low income, insecure financial status

- Educational and psychological factors
  - Low education level
  - Low self-efficacy
  - Low concerns about the illness or potential adverse effects of medication
  - Poor hypertension knowledge

- Healthcare factors
  - ***Higher payment for medication***
  - ***Poor patient provider relationship***
  - Complex medication regime
  - Multiple dosing regimen
  - Fewer health-care provider visits
  - Specialised health-care use
  - Dissatisfaction with the communication of health-care providers

Box 2.2. Factors associated with non-adherence to antihypertensive medication.
The three factors most consistently identified via systematic review are highlighted (van der Laan, Elders et al. 2017).

In conjunction with the British and Irish Hypertension Society (BIHS), NICE now recommends a structured step-wise approach to prescribing in hypertension, which aims to improve BP control in primary care (Figure 2-7). The PATHWAY 2 study has provided much needed evidence to guide the choice of drug at step 4 of the pathway, with spironolactone performing superiorly to both bisoprolol and doxazosin (and all three drugs significantly better than placebo) in a randomised, double-blind, crossover trial (Williams, MacDonald et al. 2015). When reviewing antihypertensive medication, aliskiren, a direct renin inhibitor, should also be considered. Aliskiren has been shown to

lower blood pressure to a similar degree to ACEi and ARB drugs and is usually well tolerated (may cause diarrhoea) (Pantzaris, Karanikolas et al. 2017). However, the ALTITUDE (ALiskiren Trial In Type 2 diabetes Using cardiovascular and renal Disease Endpoints) trial, which assessed the effect of adding aliskiren to an ACEi or an ARB, had to be prematurely terminated due to an increased number of adverse events (renal dysfunction, hyperkalaemia, and hypotension) with no outcome benefit (Parving, Brenner et al. 2012). The use of aliskiren is not recommended routinely in the management of hypertension, and should only be used under specialist supervision, and certainly not in conjunction with ACEi or ARB medication (Agency 2012). Protocol driven prescribing in hypertension has gone a long way to improve and standardise patient care, but as discussed below, this approach does not fully address the different mechanisms underlying hypertension or the need for individualised treatment strategies.

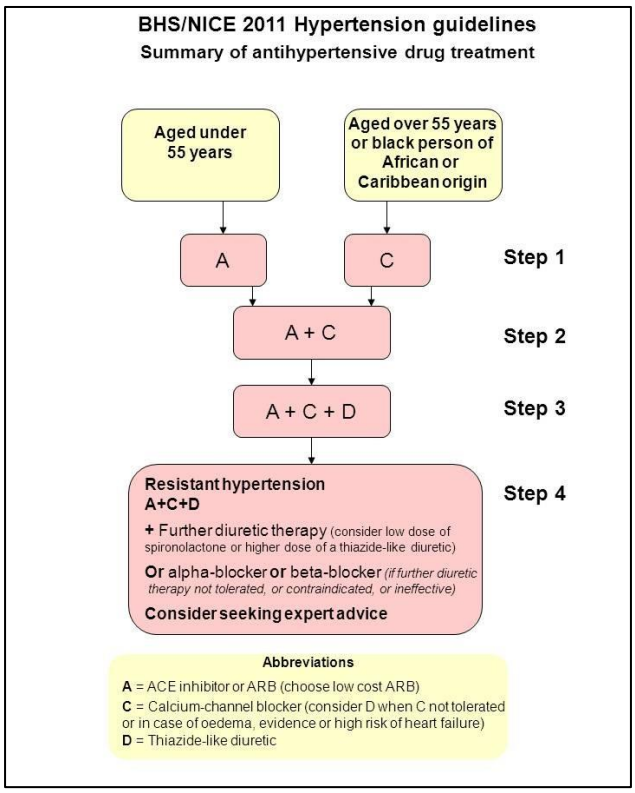


Figure 2-7. NICE Hypertension Guidelines 2011: Summary of antihypertensive drug treatment. (National Clinical Guideline 2011)

2.2.2.3 Mechanisms underlying treatment resistant hypertension

Failure to achieve BP control may be attributable to causes of secondary hypertension and inadequate pharmacotherapy, but there remains a tranche of patients with refractory hypertension, who are adherent to medication and have no evident, treatable secondary cause, but remain hypertensive. The question remains as to what is driving up blood pressure in these individuals. Treatment resistant hypertension is multi-factorial, with resistant patients more likely to be older, obese, black, and have a higher prevalence of cardiovascular disease, diabetes mellitus, OSA and chronic kidney disease

than those who respond to medication (Borrelli, De Nicola et al. 2013, Calhoun, Booth et al. 2014, Hwang, Dietrich et al. 2017).

Thinking more mechanistically, Hwang et al. propose that refractory hypertension is likely to be driven by several key factors; excess fluid retention (e.g. due to a high salt diet, obesity or CKD), hyperaldosteronism due to activation of the RAAS (which will exacerbate fluid retention), activation of the sympathetic nervous system (causing RAAS activation and increased vascular resistance), and vascular remodelling and arterial stiffness (may give isolated systolic hypertension) (Hwang, Dietrich et al. 2017). There is conflicting evidence over which of these factors is most important. The pronounced response to spironolactone in the PATHWAY-2 study may suggest that most patients are likely to have volume expansion and low-renin hypertension, (Williams, MacDonald et al. 2015), however, there is evidence to suggest that raised sympathetic tone, rather than fluid overload, may be the prevalent factor in resistant hypertension, with data from Dudenbostel et al. showing that individuals with resistant hypertension have higher urinary normetadrenaline levels, clinic and ambulatory heart rate, pulse wave velocity and systemic vascular resistance, and lower heart rate variability, than those with controlled blood pressure (Dudenbostel, Acelajado et al. 2015). Thinking about a multifactorial condition such as hypertension in this way is always going to be an over simplification, and these physiological systems interact on many levels, but it may be a helpful model to consider when planning fourth line treatment for individual patients. Figure 2-8 shows a potential patient-centred, physiology driven, approach to the pharmacological management of resistant hypertension. In a world of evolving interventional therapies for hypertension, the blood pressure phenotype of a patient may become increasingly important to appropriately direct more invasive treatments.

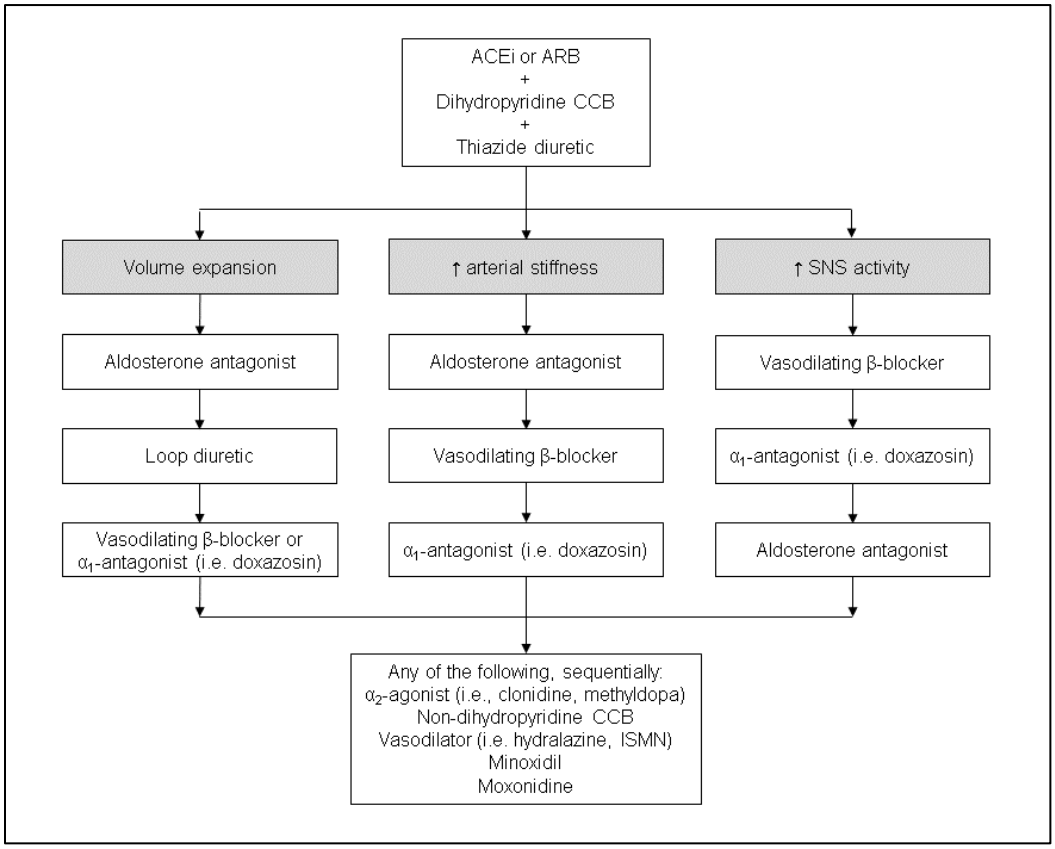


Figure 2-8. Possible mechanism-based treatment algorithm for treatment-resistant hypertension.

The recommendation is for a thiazide or thiazide-like diuretic, preferably chlorthalidone or alternatively indapamide or twice-daily hydrochlorothiazide. ACEi; angiotensin converting enzyme inhibitor, CCB; calcium channel blocker, SNS: sympathetic nervous system, ISMN; isosorbide mononitrate. Figure adapted from Hwang et al. (Hwang, Dietrich et al. 2017).

2.2.3 Prevalence of resistant hypertension

Given the frequency of pseudo-resistance the true prevalence of primary drug resistant hypertension has proven difficult to quantify, but in a comprehensive review of the data Carey estimated a prevalence of pharmacological resistance to treatment for hypertension of around 14% (Carey 2013).

The reported prevalence of resistant hypertension is extremely variable with data ranging from 10-30% in different observational cohorts (Calhoun, Jones et al. 2008, de la Sierra, Segura et al. 2011, Egan, Zhao et al. 2011, Persell 2011, Barochiner, Alfie et al. 2013), with true refractory hypertension affecting only 5-10% of patients referred to a specialist hypertension clinic for uncontrolled BP (Cai and Calhoun 2017). Retrospective analysis of patients in large clinical antihypertensive medication trials suggesting drug resistance in as many as 35% of those enrolled hypertensives (Myat, Redwood et al. 2013), whilst the recent data from Israel looking at the prescribing and dispensing of antihypertensive medication would reduce this rate to only ~2% once sub therapeutic pharmacotherapy has been taken into account (Weitzman, Chodick et al. 2014). Two

studies examining the USA National Health and Nutrition Examination Survey (NHANES) estimated the prevalence of resistant hypertension to be between 12.8 and 28% amongst treated hypertensive patients (Egan, Zhao et al. 2011, Persell 2011). The data from Jung et al. showed a prevalence of resistant hypertension of 9.6% amongst referrals to a specialist hypertension clinic (Jung, Gechter et al. 2013). The prevalence of resistant hypertension is significantly higher in high risk groups, for example, ~25% of patients with CKD have drug resistant hypertension (Borrelli, De Nicola et al. 2013). Amongst the 14,809 Participants in the REasons for Geographic And Racial Differences in Stroke (REGARDS) study who were receiving antihypertensive medications, only 78 (0.5%) had refractory hypertension with BP ≥140/90 mmHg on ≥5 classes of antihypertensive drugs (Calhoun, Booth et al. 2014).

A large cohort of 68,045 treated hypertensive patients from the Spanish Ambulatory Blood Pressure Monitoring Registry, described a prevalence of resistant hypertension of 12.2%, however, differentiating between true drug resistant hypertension and pseudo-resistance is vital (de la Sierra, Segura et al. 2011). In the Spanish Registry 37.5% of those initially diagnosed with resistant hypertension had white-coat hypertension with normal pressures on ABPM, and more recent data from the same group increased this proportion to 40% (de la Sierra, Banegas et al. 2012). In light of these findings, the latest NICE guidance recommends the use of ABPM in the diagnosis of hypertension (see Table 2-1) (National Clinical Guideline 2011).

2.2.4 Consequences of hypertension

Hypertension is often referred to as a silent killer, because whilst most suffers are asymptomatic, the condition is an established risk factor for cardiovascular disease (CVD). The World Health Organisation estimates that 11% of all disease burden in developed countries is due to high BP, and 50% of all coronary heart disease (CHD) and 75% of all stroke in these countries can be attributed to a systolic BP of >115mmHg (WHO 2013). This finding was reaffirmed by the INTERHEART study which reported that 22% of myocardial infarcts in Western Europe were due to hypertension (Yusuf, Hawken et al. 2004). This cardiovascular risk is incremental; with each 20mmHg rise in SBP or 10mmHg rise in DBP above 115/70mmHg giving a doubling in the risk of death from CVD in adults aged 40-69 (Lewington, Clarke et al. 2002). Treating hypertension is, however, beneficial; a reduction in DBP of 5/7.5/10 mmHg gives a 34/46/56% reduction in stroke and a 21/29/37% reduction in CHD (MacMahon, Peto et al. 1990). Similarly, a sustained reduction in DBP of 5-6mmHg over 5years reduced the relative risk of stroke and CHD by 42% and 14% respectively (Collins, Peto et al. 1990).

The financial and logistical implications of managing hypertension are huge, accounting for 12% of primary care consultations and £1 billion in drug costs in 2006 (National Clinical Guideline 2011), but it is estimated that the NHS could save around £97.2 million from reduced complications such as stroke, heart failure and renal failure if BP could be reduced to less than 140/90mmHg (Lloyd, Schmieder et al. 2003) .

2.2.4.1 Prognosis in resistant hypertension

Unfortunately, this patient group has a particularly high risk of serious cardiovascular complications including development of stroke, heart failure and chronic renal disease. Identifying those most at risk remains key; those with drug resistant hypertension are nearly 50% more likely to suffer a CVD event than those on <3 antihypertensive medications (Daugherty, Powers et al. 2012). In the Reduction of Atherothrombosis for Continued Health (REACH) registry of 53,530 patients, the prevalence of resistant hypertension was 12.7%, and the condition conferred a significantly increased risk of cardiovascular death, myocardial infarction, or stroke at 4 years (Kumbhani, Steg et al. 2013). Hospitalisations due to congestive heart failure were also increased. A sub-analysis of the ALLHAT (Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial) study identified 1870/14684 (13%) participants with resistant hypertension. When compared with study participants without resistant hypertension, participants with resistant hypertension had a 44%, 57%, 23%, 88%, 95%, and 30% higher risk of incident coronary heart disease, stroke, peripheral artery disease, heart failure, end-stage renal disease, and all-cause mortality, respectively, after adjustment for traditional risk factors (Muntner, Davis et al. 2014). Risk factors for resistant hypertension include diabetes, renal impairment, obesity and advancing age, all of which are increasing in prevalence in the UK (de la Sierra, Segura et al. 2011, de la Sierra, Banegas et al. 2012, Calhoun, Booth et al. 2014).

Previously, patients failing to achieve BP control despite pharmacological and life-style interventions have been left with few therapeutic options. There have been no new classes of anti-hypertensive medication approved for clinical use in recent years (sacubitril valsartan not yet licenced for use in hypertension), and research into drug development in this field is now a vastly expensive and time consuming process (Garber 2009) . The development of novel techniques such as renal denervation, baroreflex stimulation and carotid body modulation, offers new avenues for these high-risk individuals, in what is now a rapidly developing field.

2.3 Renal Denervation: A novel therapy for resistant hypertension

2.3.1 The Evolution of Renal Denervation

At the beginning of the 20th century, there was no treatment available for hypertension until progress was made with the introduction of surgical sympathectomy in 1935 (Esler 2015). Surgical sympathectomy was used into the late 1950s as a treatment for hypertension, and successfully lowered blood pressure, but with significant side-effects, including orthostatic and post-prandial hypotension, syncope and sexual dysfunction (Whitelaw and Smithwick 1951, Morrissey, Brookes et al. 1953, Longland and Gibb 1954, Esler 2015). Sympathetic ganglionic blockade, starting with drugs such as hexamethonium discovered by William Paton in the early 1950s (Paton 1982), was employed to replace surgical sympathectomy, and lowered blood pressure without exposure to surgical risks, but with similar significant side effects, including orthostatic hypotension, syncope, constipation, mydriasis and impotence (Fisher and Paton 2012, Esler 2015). Building on this concept of sympathoinhibition, anti-adrenergic drugs, acting both centrally (methyldopa, clonidine) and peripherally (α and β adrenergic blockers) were developed over the following decades, and their use, in combination with diuretics and vasodilators, and then more recently calcium channel blockers and drugs targeting the renin angiotensin aldosterone system (RAAS) have become the mainstay of anti-hypertensive therapy (see Figure 2-7 (National Clinical Guideline 2011, Mancia, Fagard et al. 2013, Esler 2015)).

In the 21st century, the increasing support for the concept of neurogenic hypertension, particularly the important link between afferent and efferent renal signalling and elevated sympathetic tone in the development and maintenance of hypertension, has led researchers to renal denervation as a therapeutic strategy for patients with resistant hypertension. In the 1970s and 80s, the efficacy of RDN in the reduction of sympathetic nerve activity and BP was established in animal models (Liard 1977, Katholi, Winternitz et al. 1982, Winternitz and Oparil 1982, Katholi 1983, Lee and Walsh 1983). In humans, radical nephrectomy is associated with normalisation of muscle sympathetic nerve activity (MSNA) in hypertensive patients with CKD, and disruption of afferent signals from the renal nerves, which drive central up-regulation of the sympathetic response, is believed to contribute to this reduction in vasomotor tone (Converse, Jacobsen et al. 1992, Phillips 2005). In recent years, improvements in endovascular ablation techniques, a decline in the development of new pharmacological therapies for hypertension, and the growing appreciation of the neurogenic hypertension paradigm, have refocused research. This has led to the development of novel non-pharmacological interventions targeting the sympathetic nervous system. One such intervention is catheter-delivered renal denervation.

2.3.1.1 Catheter development and pre-clinical studies

RDN developed as a percutaneous endovascular technique that achieves denervation using a radiofrequency ablation catheter. A catheter is used to apply radio-frequency energy to the inner wall of each renal artery, disrupting the renal nerves. The hypothesis was, that by disrupting the efferent sympathetic nerves which stimulate vasoconstriction with reduced RBF, renin release and sodium retention, BP would be

reduced (DiBona and Esler 2010). Stimulation of the afferent renal nerves in hypertension due to increase arterial pressure, microvascular damage, angiotensin II mediated hypoperfusion, hypoxia and inflammation, may act to drive up blood pressure through a reflex increase in SNA (Koeners, Lewis et al. 2016). Thus, targeted ablation of both the afferent and efferent renal nerves will interrupt a pathological positive feedback loop in which the kidneys are, essentially, driven to reduce their own blood supply, whilst avoiding the adverse effects of denervating other structures observed in earlier studies (i.e. target specific ablation). Denervation of the renal efferent nerves results in a shift in the pressure-natriuresis curve to the left, encouraging renal sodium excretion, and renal afferent denervation disrupts the pro-hypertensive sympathoexcitation seen as a result of renal afferent hyperactivity (Sobotka, Mahfoud et al. 2011).

In 1859, Claude Bernard demonstrated that cutting the greater splanchnic nerve (includes denervation of the kidney) resulted in an ipsilateral diuresis, and that stimulation of the distal end of the nerve (renal nerve stimulation) gives an antidiuresis (Bernard 1859, DiBona and Esler 2010). Since then, renal denervation has been shown to prevent or delay the onset of hypertension in a wide arrange of animal models, including the spontaneously hypertensive rat, Goldblatt one-kidney, one-clip and two-kidney, one-clip rats, the DOCA-Na^+ rat (deoxycorticosterone acetate), and the DOCA dog models (DiBona and Esler 2010). In the 1980s, Katholi et al. demonstrated a reduction in plasma norepinephrine levels following renal denervation in the one-kidney, one-clip Goldblatt rat model, indicating an effect of RDN on sympathetic tone (Katholi, Winternitz et al. 1982). More recently, Hart et al. reported a reduction in arterial pressure, renal norepinephrine content and lumbar SNA following renal denervation in conscious spontaneously hypertensive rats (Hart, McBryde et al. 2013).

Three factors governed the development of endovascular RDN for use in clinical practice (Esler 2015).

1. Evidence for sympathoexcitation in human hypertension, including raised renal SNA (see Section 2.1.2.1) (Goldstein, Horwitz et al. 1983, Esler, Jennings et al. 1986, Yamada, Miyajima et al. 1989, Esler and Kaye 2000).
2. Evidence showing a blood pressure reduction following surgical renal denervation in animal models (Liard 1977, Katholi, Winternitz et al. 1982, Winternitz and Oparil 1982, Katholi 1983, Lee and Walsh 1983, DiBona and Esler 2010, Hart, McBryde et al. 2013).
3. The anatomy of the renal nerves which lie in a reticular network within the adventitia of the renal artery, making them amenable to endovascular ablation (Sakakura, Ladich et al. 2014).

Levin and Gelfand were the first to patent a renovascular catheter for the ablation of renal nerves as a treatment for hypertension in 2002 (Esler 2015). This patent was acquired by the start-up company Ardian (California, USA) under whose auspices the first pre-clinical study, using a custom designed catheter, was carried out in a porcine model, demonstrating safety and efficacy for renal nerve ablation (Rippy, Zarins et al. 2011).

The first catheter to become commercially available for clinical use was the Symplicity Flex catheter (Medtronic, Minneapolis, Minnesota, USA, previously Ardian). The

catheter had a single radio-frequency electrode at its tip, which could be angulated to administer a series of 4-8, 2-minute-long ablations distributed in a spiral pattern going from the distal to proximal aspect of the renal artery. Software within the catheter console monitored the arterial wall temperature and resistance to aid the successful administration of therapies (see Figure 2-9) (Krum, Schlaich et al. 2009). The duration of the RDN procedure (requiring multiple, bilateral ablations) and the technical challenge of achieving adequate circumferential denervation using the Symplicity Flex catheter, and the financial incentive of entering the market in a novel procedure, have driven the development of second generation devices using multi-electrode spiral, multi-pronged basket, or balloon mounted technologies. At the peak of the RDN boom in early 2014, there were over 50 RDN catheters in development.

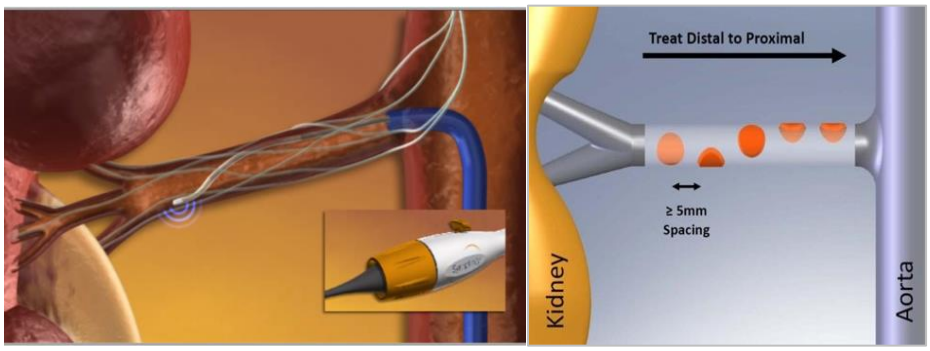


Figure 2-9. Renal denervation using the Symplicity Flex catheter.
(http://www.medtronicrdn.com).

2.3.2 Renal Denervation: the early clinical trials

In the first published clinical RDN case, Schlaich at al. describe a patient in which BP was successfully reduced from 161/107 mmHg at baseline, to 141/90 mmHg at 30 days and to 127/81 mmHg at 12 months after ablation (Schlaich, Sobotka et al. 2009). In this case whole-body NA spillover was reduced by 42%, elevated baseline MSNA returned to normal levels, cardiac baroreflex sensitivity improved and cardiac magnetic resonance imaging (MRI) showed a reduction in the left ventricular mass following RDN (Schlaich, Sobotka et al. 2009).

2.3.2.1 Symplicity HTN-1

Proof of concept for RDN, and an initial demonstration of procedural safety, was achieved in the Symplicity HTN-1 study (Krum, Schlaich et al. 2009). The initial cohort of 45 patients with a mean office blood pressure of 177/101, had an average office BP reduction of -22/-11 (n=26) and -27/-17 (n=9) mmHg at 6 and 12 months post RDN respectively. Renal NA spillover was assessed in a subgroup of 10 patients and was reduced by 45% following RDN. At 36 months post-procedure, 93% of the 88/153 patients who remained under follow-up had a reduction in office SBP of ≥10 mmHg (Krum, Schlaich et al. 2014). The mean change in office BP was -32/-14 mmHg, sustained out to three years post-denervation (Figure 2-10).

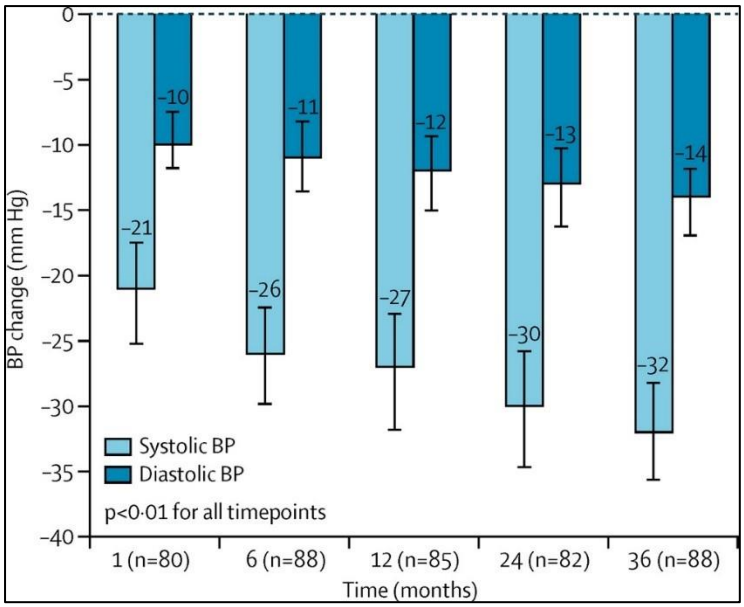


Figure 2-10. Change from baseline in office blood pressure (BP) in patients who completed 36 months of follow-up in the Symplicity HTN-1 study.
(Krum, Schlaich et al. 2014)

2.3.2.2 Symplicity HTN-2

In Symplicity HTN-2, a highly-publicised multi-centre randomised controlled trial of RDN in patients with resistant, an office BP reduction of -32/-12mmHg (-33/-11mmHg difference versus control, p<0.0001) was observed at 6-months post RDN, again with no significant sustained adverse sequelae (Esler, Krum et al. 2010). Symplicity HTN-2 was a cross-over study with the control group offered RDN 6 months after the treatment group (Esler, Krum et al. 2012). As can been seen from Figure 2-11, the RDN group had a significant reduction in office BP after six months, not seen in the control group, with the control group showing a similar BP reduction following cross-over to RDN therapy after six months (Esler, Krum et al. 2012).

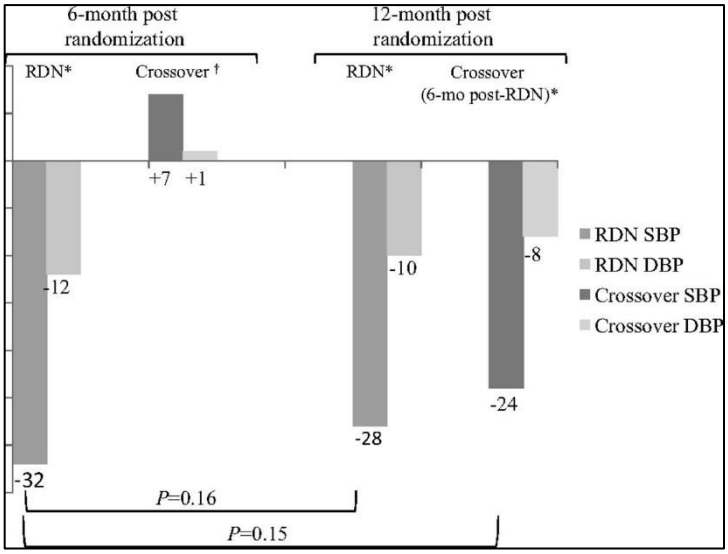


Figure 2-11. Change in office-based blood pressure after 1-year follow-up in Symplicity HTN-2 study.

*P<0.001 for SBP and DBP change after renal denervation; † p=0.026 for SBP change from baseline and p=0.066 for DBP change from baseline for the crossover group before denervation at 6 months (Esler, Krum et al. 2012).

There have been several technical issues raised regarding the Symplicity studies that should be highlighted (Krum, Schlaich et al. 2009, Esler, Krum et al. 2010); for example, these studies, and several others in the field, report clinic BP (or office BP) as their primary outcome measure. Ambulatory BP monitoring (ABPM) is now recommended as the gold standard in the diagnosis of hypertension, and the use of office BP may have resulted in the overestimation of BP and the inclusion of 'white-coat' hypertensives, rather than those with true 'resistant' hypertension, in these studies. It is also of note that 35% of the control group, who received standard medical therapy, in Symplicity HTN-2 had a >10mmHg reduction in systolic BP at 6 months (Esler, Krum et al. 2010); an observation that could be explained by improved compliance with medication, or optimisation of medications, following enrolment in the study, and which must also be transposed to the treatment group (a Hawthorne effect (McCambridge, Witton et al. 2014)). Changes in drug regimens pre- and post RDN were discouraged in the Symplicity studies, but these did occur and must be factored into data interpretation. Given the gain in emphasis for the use of aldosterone antagonists in the most recent NICE Hypertension Guidelines (National Clinical Guideline 2011), now supported by evidence from the PATHWAY-2 study (Williams, MacDonald et al. 2015), and although RDN remained effective in Symplicity HTN-2, it is notable that only 17% of patients in Symplicity HTN-2 were on spironolactone and therefore many participants were receiving sub-optimal medical management.

2.3.2.3 EnligHTN-I & II

The main competitor rivalling the Medtronic Symplicity Flex catheter was the EnligHTN catheter produced by St Jude Medical (St Paul, Minnesota, USA). The EnligHTN catheter employs a basket design with four electrodes which can ablate simultaneously, aiming to target circumferential ablation and speed up the procedure (Worthley, Tsioufis et al.

2013). EnligHTN-I, a safety and feasibility study, reported an office BP reduction of 26/10 mmHg six months post-RDN, with 80% of participant s (n=45) achieving an office SBP reduction of ≥10 mmHg (Worthley, Tsioufis et al. 2013). The study also reports ABPM outcomes, with a significant -10/-6 mmHg change in mean 24hr BP. The BP reductions seen in EnligHTN-I persisted out to 24 months with falls of -29/-13 mmHg and -13/-7 mmHg for office and ambulatory BP respectively.

EnligHTN-II recruited patients into three sub groups: severe hypertension (office SBP ≥160 mmHg and estimated glomerular filtration rate (eGFR) ≥45 mL/min per 1.73 m^2), moderate hypertension (office SBP ≥140–159 mmHg and eGFR ≥45 mL/min per 1.73 m^2) and hypertension with renal impairment (office SBP ≥140 mmHg and eGFR ≥ 15 mL/min per 1.73 m^2). Interestingly, the study recruited both patients with drug resistant hypertension and those with drug intolerance, unable to take three antihypertensive medications (Lobo, Saxena et al. 2015). Data have been published for the severe hypertension group, reporting significant reductions in office BP (18.2/8.5 mmHg) and ABPM (7.9/4.8 mmHg) 6 months after the RDN procedure (Lobo, Saxena et al. 2015).

2.3.2.4 Renal denervation in acceleration

On the back of the positive outcomes seen in Symplicity HTN-1 &2 and EnligHTN-I, the field of renal denervation went into rapid acceleration, with multiple independent clinical groups publishing outcome data, and industry embracing the field with the development of other radiofrequency ablation catheters and the use of alternative technologies to achieve renal nerve ablation (e.g., focussed ultrasound or perivascular chemoablation). The range of catheters under development is summarised in Table 2-3 with the results from clinical trials (where available) summarised in Table 2-4. This list is not exhaustive but covers those devices with a significant, published, clinical evidence base, other devices under development and/or clinical trial include the Allegro and Iberis (AngioCare Medical, Shanghai, China), Redy (Renal Dynamics, Stuttgart, Germany) and Chilli II (Boston Scientific Corporation, San Jose, California) radiofrequency ablation catheters, the TIVUS ultrasonic ablation catheter (CardioSonic, Tel Aviv, Israel) and the targeted sympathetic mapping/ablation catheter, SyMapCath I (SyMap Medical, Suzhou, China). The results of these studies largely support the antihypertensive effect of RDN seen in Symplicity HTN-1&2, but not universally so, with some of the independent groups reporting little or no blood pressure effect from the procedure. Published data from the Bristol CardioNomics group and St Bartholomew's Hospital in London, and results from other European centres suggest that in contrast to Symplicity HTN-2, only up to around 50% of patients achieve a clinically significant reduction in BP with RDN (Brinkmann, Heusser et al. 2012, Vase, Mathiassen et al. 2012, Kaltenbach, Franke et al. 2013, Hameed, Pucci et al. 2015, Rohla, Nahler et al. 2015, Burchell, Chan et al. 2016). Notably, Brinkmann et al. reported no significant reduction in mean BP, MSNA, heart rate variability (HRV) and blood pressure variability (BPV), or increase in sympathetic or cardiac baroreflex sensitivity (BRS), following RDN in their patients (Brinkmann, Heusser et al. 2012). Their study was criticised for the inclusion of patients with relatively low baseline BP and MSNA levels, and for the reporting of supine beat-to-beat BP results, rather than office BP, or more preferably ABPM. One interpretation was that Brinkmann et al.'s results suggested that patients with more severe hypertension are more likely to respond to RDN, a finding reported by other groups (Kandzari, Bhatt et al. 2015, Rohla,

Nahler et al. 2015, Burchell, Chan et al. 2016). However, similar findings have been published by the Bristol CardioNomics group, with preliminary data from a small study of 8 patients, showing no overall reduction in BP or MSNA 1 and 6 months post-RDN ($p<0.05$), with no correlation between BP reduction an MSNA in the 4 patients who did respond to treatment (Hart, McBryde et al. 2013). Interestingly, in this translational study, a reduction in BP and SNA was seen in all of the rats (n=7) which underwent surgical renal denervation, and baroreflex sensitivity was consistently improved in both animals and humans, even when BP remained unchanged in the human subjects (Hart, McBryde et al. 2013). It remained evident that RDN was not a panacea, and it was still unclear whether patients failed to respond due to inappropriate patient selection for RDN, or technical limitations with the procedure itself resulting in incomplete denervation.

2.3.2.5 Data from renal denervation registries

As renal denervation moved into clinical practice, several registries were established to collate safety and outcome data. The largest of these is the Global Symplicity Register which reported a response (office BP reduction ≥10 mmHg) rate of 67% in 998 patients 6 months post-RDN (Bohm, Mahfoud et al. 2015). The UK Renal Denervation Affiliation has reported an office BP reduction of 22/9 mmHg ($p<0.001$) with a 65% response rate, in a cohort of 246 patients from 16 centres (Sharp, Hameed et al. 2015). The TREND registry of 407 patients from 14 centres in Austria, reported an office BP responder rate of 69% (128 of 185 patients) (Zweiker, Lambert et al. 2016). The ALSTER, Heidelberg and Greek registries also report real-world data, with better response rates of 76% (n=93), 73% (n=63) and 85% (n=79), respectively (Kaiser, Beister et al. 2014, Vogel, Kirchberger et al. 2014, Tsioufis, Ziakas et al. 2017). The recently published Swedish registry of 252 patients reported an office SBP response rate of only 58% (Volz, Spaak et al. 2018).

Devices (producer)	Characteristics	Major Trials (n)
Radiofrequency ablation		
Symplicity™ Renal Denervation System (Medtronic, Santa Rosa, CA, USA)	Non-occlusive flexible catheter with a single electrode tip	Symplicity HTN I (152) completed Symplicity HTN II (106) completed Symplicity HTN III (530) completed
EnligHTN™ Multi Electrode Renal Denervation System (St. Jude Medical, St. Paul, MN, USA)	Occlusive, over the wire balloon catheter with embedded multi-electrodes	EnligHTN I (47) completed EnligHTN II (500) full results awaited EnligHTN III (39) completed EnligHTN IV (4) sham RCT, terminated
OneShot™ Renal Denervation System (Covidien, Campbell, CA, USA)	Irrigated, helical over the wire balloon catheter	RHAS (12) completed RAPID (50) follow-up
V2 Renal Denervation System™	Over the wire variable size balloon catheter	REDUCE-HTN (150) completed

(Vessix Vascular, Boston Scientific, Marlborough, MA, USA)	with embedded bipolar electrodes	REDUCE-HTN: REINFORCE (100) recruiting, uncontrolled HTN off-medications, vs masked procedure (renal angiogram)
Celsius® ThermoCool® Renal Denervation Catheter (Biosense Webster, CA, USA / Cordis)	Irrigated, multi-electrode	RENABLATE (30) completed RENABLATE-II (14) completed
Symplicity Spyral™ Renal Denervation System (Medtronic, Santa Rosa, CA, USA)	Non-occlusive, multi-electrode helical catheter	SPYRAL HTN-ON MED (100) preliminary data published SPYRAL HTN-OFF MED (170)preliminary data published, both sham controlled
Ultrasonic ablation		
Paradise™ Renal Denervation System (ReCor Medical, Palo Alto, CA, USA)	Endovascular balloon catheter combined with a US-emitting transducer and cooling system	REDUCE (11) completed REALISE (20) completed ACHIEVE (96) follow-up REQUIRE (140) recruiting, sham controlled RADIANCE-HTN (292) recruiting, sham controlled (SOLO: off medications, TRIO: triple fixed dose combined medication)
Surround Sound System (Kona Medical, Bellevue, WA, USA)	Externally delivered focussed ultrasound	WAVE I, II &III (69) completed WAVE IV (81) stopped prematurely
Tissue-directed pharmacological ablation		
Peregrine Ablation System (Ablative Solutions, Kalamazoo, MI, USA)	Three-needle device for the peri-adventitial injection of micro-doses of ethanol	Fischell et al.
Bullfrog Microinfusion Catheter (Mercator MedSystems, Emeryville, CA, USA)	Balloon sheathed micro-needle	TREND-I (7) completed

Table 2-3. Catheters developed for renal nerve ablation using radiofrequency, ultrasound and pharmacological ablation technologies.
RDN; renal denervation, US: ultrasound. (Gulati and White 2013, Heeger, Kaiser et al. 2013)

Trial	Device(s)	No. of Pts	Baseline BP (mmHg)	Change in oBP at 6 months (mmHg)	Response rate at 6 months (%)	Comment
Symplicity HTN-1(Krum, Schlaich et al. 2009)	Symplicity Flex	45	177/101	-22/-11	87	Single RF electrode on flexible catheter tip
Symplicity HTN-2 (Esler, Krum et al. 2010)	Symplicity Flex Control	49 39	178/96 178/97	-32/-12 1/0	84 -	Randomised controlled study with cross-over to RDN at 6 months
Symplicity HTN-3 (Bhatt, Kandzari et al. 2014)	Symplicity Flex Sham	364 171	180/97 180/99	-14/-7 -12/-5	58 49	Sham controlled trial, showing no benefit of RDN over medical therapy
SPYRAL HTN-ON MED (Kandzari, Bohm et al. 2018)	Spyral Sham	38 42	165/100 164/103	-9/-5 -3/-2	- -	Sham controlled trial, standardised medication regime with objective quantification of adherence, oBP data shown, ABPM used as primary endpoint.
SPYRAL HTN-OFF MED (Townsend, Mahfoud et al. 2017)	Spyral Sham	38 42	162/100 161/102	-10/-5 -2/0	- -	Sham controlled trial, patients off antihypertensive medication, 3-month oBP data shown, ABPM used as primary endpoint.
Symplicity HTN-Japan (Kario, Ogawa et al. 2015)	Symplicity Flex Control	22 19	181/ 179/	-17/-6 -8/1		No significant difference between treatment groups, terminated early due to HTN-3 results.
Brinkmann et al. (Brinkmann, Heusser et al. 2012)	Symplicity Flex	12	157/85	0/0	25	Reports supine beat-to-beat BP data collected 3-6 months post RDN, also no effect on MSNA.
DENERHTN (Azizi, Sapoval et al. 2015)	Symplicity Flex Control	48 53	160/93 156/91	-15/-9 -10/-6	42* *21**	Assessed RDN added to standardised stepped antihypertensive treatment. *% with ≥20 mmHg reduction in daytime ABPM
Hameed et al. (Hameed, Pucci et al. 2015)	Symplicity Flex	34	185/103	-15/-6	51	Medication adherence confirmed by directly observed medication administration.
PRAGUE-15 (Rosa, Widimsky et al. 2016)	Symplicity Flex Spironolactone	52 54	159/92 155/89	-13/-8* -11/-6*	57* 50*	RDN vs addition of spironolactone, outcome data is for 12-month post-RDN
EnligHTN I (Worthley, Tsioufis et al. 2013)	EnligHTN	45	176/96	-26/-10	80	Basket with four RF electrodes

EnligHTN III (Worthley, Wilkins et al. 2017)	EnligHTN	39	174/93	-25/-7	81	2nd generation EnligHTN catheter
INSPIRED (Jacobs, Persu et al. 2017)	EnligHTN Control	6 9	173/103 159/95	-12/-8 8/2		Quality of life and adherence similar between groups
RHAS (Ormiston, Watson et al. 2013)	OneShot	9	186/92	-34/-13	75	Irrigated RF balloon catheter
RAPID (Verheye, Ormiston et al. 2015)	OneShot	50	162/96	-20/-8	62	
REDUCE-HTN (Sievert, Schofer et al. 2015)	Vessix V2	146	182/100	-25/-10	76	Balloon catheter with 4-8 bipolar RF electrodes on surface
RENABLATE-II, 2013 (NCT02095691)	Celsius ThermoCool	14		-11/-1	50	Limited data only available via clinicaltrials.gov
Fischell et al. (Fischell, Ebner et al. 2016)	Peregrine	18	175/-	-25/-12	75	Three-needle device for the peri-adventitial injection of micro-doses of ethanol
REALISE (Montalescot, Cluzel et al. 2014)	Paradise	20	167/-	-41/-		Reduction in SBP in *responders*, abstract only
RADIANCE HTN-SOLO (Azizi, Schmieder et al. 2018)	Paradise Sham	74 72	155/100 154/99	-11/-6 -4/-1	- -	Sham controlled trial, off medication, 2-month oBP data shown, ABPM used as primary endpoint.
WAVE I, II, III (Neuzil, Ormiston et al. 2016)	Surround Sound	69	180/98	-25/-9	75	
WAVE IV (Schmieder, Ott et al. 2018)	Surround Sound Sham	42 39	181/100 185/100	-13/-5 -23/-9	- -	Sham controlled, terminated early
TREND-I (Kipshidze, Sievert et al. 2017)	Bullfrog	7	189/94	-36/-1		Injection of a single dose of NW2013, a neurotropic Na^+/K^+ ATPase antagonist

Table 2-4. Summary data from clinical renal denervation studies illustrating the range of catheters in use and variable blood pressure outcome data reported.

RDN; renal denervation, RF; radiofrequency, MSNA; muscle sympathetic nerve activity, oBP; office blood pressure, BP; blood pressure, ABPM; ambulatory BP monitoring, RHAS; renal hypertension ablation system. *ABPM data shown.

2.3.3 Symplicity HTN-3

2.3.3.1 The trial

Symplicity HTN-3 was a large randomised controlled trial (RCT) undertaken in the USA with a view to obtaining FDA (Food and Drug Administration Agency) approval for RDN (Bhatt, Kandzari et al. 2014). A total of 535 patients with resistant hypertension were randomised on a 2:1 basis to either denervation with the Symplicity Flex catheter (Medtronic) or a sham procedure. The primary endpoint of the study was the change in office systolic blood pressure 6 months after the procedure in comparison to the sham control group, with a superiority margin set at 5 mmHg. The results of the study, published at the beginning of April 2014, sent shockwaves through the renal denervation community. The blinded study failed to show a benefit of RDN over the sham procedure, with both cohorts having a significant reduction in office SBP at 6-months (-14.1 ± 23.9 mmHg and -11.7 ± 25.9 mmHg respectively (both $p<0.001$, see Figure 2-12) (Bhatt, Kandzari et al. 2014). There was similarly no benefit of RDN over sham for a reduction in 24-hour ambulatory BP or conversion to nocturnal dipping status (Bakris, Townsend et al. 2014). The results of the study had a huge impact on the field, including the premature termination of EnligHTN-IV (the competing sham RCT from St Jude) amongst other studies. In the UK, RDN had been moving towards review with NICE for potential NHS funding, and this process was suspended.

2.3.3.2 The critique

The results of Symplicity HTN-3 raise into question the efficacy of RDN for the treatment of resistant hypertension. However, the design and conduct of the trial have come under substantial review and criticism, particularly by proponents of the technique (Esler 2014, Luscher and Mahfoud 2014, Pathak, Ewen et al. 2014, Kandzari, Bhatt et al. 2015, Pocock, Bakris et al. 2016, Raman, Tsioufis et al. 2017). In reviewing the major issues affecting Symplicity HTN-3, Felix Mahfoud attributed factors to three P's; Patient, Pills and Procedure (oral presentation, European Society of Cardiology, Rome, 2016). For consistency, I will review the study under these headings.

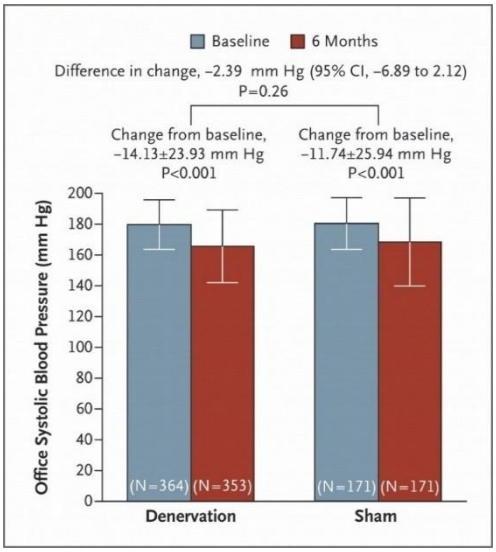


Figure 2-12. Office systolic blood pressure outcomes after renal denervation or sham procedure in the Symplicity HTN-3 study
(Bhatt, Kandzari et al. 2014).

1.1.1.1.1 Patient: Selection for Renal Denervation

When enrolling patients into studies of renal denervation (or any other intervention), it is important to thoroughly screen patients so that a definite baseline has been established; causes of pseudo-resistant hypertension may act as confounders. In Symplicity HTN-2, 109 out of 190 (56%) patients screened were eligible for RDN. In Symplicity HTN-3, tighter screening including ABPM found that 561 (39%) of 1441 patients assessed across 88 sites, were eligible for enrolment (Esler, Krum et al. 2010, Bhatt, Kandzari et al. 2014). In a review of patients attending the Specialist Hypertension Clinic at the Bristol Heart Institute, 184 underwent renal magnetic resonance or computerised tomography (CT) angiography as part of their assessment for secondary hypertension; 20% of these patients (36/184) were anatomically ineligible for RDN including 8 cases of renal artery stenosis (Burchell, Rodrigues et al. 2017). This is a slightly higher anatomical exclusion rate that the 16% (30/190) of patients with ineligible anatomy in Symplicity HTN-2, but of a similar magnitude to the 20% (179/880) anatomical exclusion rate in Symplicity HTN-3 (Esler, Krum et al. 2010, Bhatt, Kandzari et al. 2014).

Mahfoud et al. compared the reduction in office and ambulatory BP in patients with resistant and pseudo-resistant hypertension following renal denervation; whilst both groups demonstrated a reduction in office BP, only those with true resistant hypertension demonstrated a significant reduction in 24hr ABPM (-10/-5 mmHg) (Mahfoud, Ukena et al. 2013).

Verloop et al. screened 181 patients for severe resistant hypertension prior to RDN and found that 121 (67%) were ineligible for their study, due to a range of factors: 23 patients (19%) had an office SBP <160 mmHg, 26 patients (22%) showed a white-coat effect, 14 patients (12%) had a previous undetected underlying cause of hypertension (primary aldosteronism in 11), and 9 patients (7%) had ineligible renal anatomy (Verloop, Vink et al. 2013). The combined experience of the Bristol CardioNomics group

and St Bartholomew's Hospital, reported that meticulous screening of 321 patients identified only 33 individuals (10%) with true treatment resistant hypertension, suitable renal artery anatomy, and without significant excluding comorbidities (including eGFR <45 ml/min/1.73m^2 as per Symplicity HTN-2 (Esler, Krum et al. 2010)) who were eligible for RDN (Burchell, Chan et al. 2016). This is consistent with estimates that 10-15% of patients with hypertension are genuinely treatment resistant once secondary causes of hypertension, pseudo-resistant hypertension and poor medication adherence are excluded (de la Sierra, Segura et al. 2011, Persell 2011). In the case of Symplicity HTN-3, was the screening process rigorous enough to ensure a robust baseline? Enrolling patients with pseudo-resistant hypertension into RDN studies for resistant hypertension not only jeopardises the results, as these patients may not respond to RDN in the same way as the defined cohort, but also puts the patients at risk by exposing them to a procedure which may well be unnecessary or to which they will not respond.

The question also goes beyond negative screening (aiming to exclude those ineligible for the study), to positive screening, and whether it is possible to identify patients who are more likely to respond to the procedure. Sympathoexcitation or markers of renal injury would hypothetically indicate efferent or afferent renal nerve overactivity respectively and could be used to select patients who may be more likely to respond to renal nerve ablation. These autonomic or biochemical markers are not yet in clinical use but are reviewed in Section 5.6. One factor that has been highlighted from the results of Symplicity HTN-3 is that patients with isolated systolic hypertension (ISH) have a far less pronounced response to RDN that those with combined systolic and diastolic hypertension (pooled data for patients with ISH from the Symplicity HTN-3 study and the Symplicity Global Registry, (Mahfoud, Bakris et al. 2017)). This result supports findings by Ewen et al. indicates that patients with ISH and therefore lower DBP, have a restricted response to RDN (Ewen, Ukena et al. 2015). Further research is required to identify which individuals are most likely to benefit from this invasive procedure and addressing this issue through detailed autonomic profiling of patients undergoing RDN is a major aim of this thesis.

1.1.1.1.2 Pills: Medication Alteration and Adherence

There are important limitations with all of the Symplicity HTN studies surrounding the confirmation of adherence to medications and also changes in antihypertensive medication during the follow-up period (Krum, Schlaich et al. 2009, Esler, Krum et al. 2010, Bhatt, Kandzari et al. 2014).

In Symplicity HTN-2&3 there were medication changes in 23% and 39% of patients prior to 6 month follow-up respectively, however, the primary study outcomes were unaltered if patients with medication changes were removed from analyses (Esler, Krum et al. 2010, Bhatt, Kandzari et al. 2014, Kandzari, Bhatt et al. 2015). The standardised stepped-care antihypertensive treatment (SSAHT) regime used in the DENER-HTN study demonstrates that this issue can be well managed, although adequate patient support and infrastructure is required (Azizi, Sapoval et al. 2015). In this study patients treated with SSAHT plus RDN had a greater reduction in daytime ambulatory BP than those treated with SSAHT alone (-15.8 mmHg vs -9.9 mmHg).

In the Symplicity HTN-3 trial, only 1 in 5 patients had received a trial of spironolactone, and 22% of patients had a medication change 2–6 weeks before the study started

(Yerasi, Baker et al. 2015). The PRAGUE-15 study showed that spironolactone, when tolerated and continued, is more effective at reducing BP than RDN (Rosa, Widimsky et al. 2016). The run-in period prior to RDN should also be considered; in Symplicity HTN-3 patients were only required to be on a stable drug regimen for two weeks prior to baseline assessments and it is therefore possible that medication changes could have influenced the data if there was an inadequate wash-in/wash-out period. An eight-week period on stable medication should be required to ensure that any intervention is not confounded by a time-dependent drug effect (Lobo, de Belder et al. 2015).

Symplicity HTN-3 did not simply show a failure to alter BP, it demonstrated a significant reduction in office SBP in both RDN and sham groups (Bhatt, Kandzari et al. 2014). Of note, in Symplicity HTN-2 35% of control subjects had a ≥10 mmHg reduction in office SBP six months post RDN (Esler, Krum et al. 2010). This decrease in BP may be explained by an improvement in medication adherence. The phenomenon of a 'placebo' effect due to enrolment in a clinical study (also known as the Hawthorne effect) is well established (McCambridge, Witton et al. 2014) and it is likely that the 8 study contact points between screening and 6 month follow-up in Symplicity HTN-3 provided greater patient support than standard medical care (Kandzari, Bhatt et al. 2015). Yerasi et al. have undertaken an interesting assessment of 45 patients who were screened for, but ultimately not included in, the Symplicity HTN-3 study. In this group, 6-8 months after previous study contact, only 20% had resistant hypertension with 60% of patients having controlled BP (Yerasi, Baker et al. 2015). This partly reflects the fact that some of the patients had been excluded due to controlled BP on screening but does emphasise the challenge of comparing hypertension control in daily life with BP regulation in clinical trials.

Kandazari et al. highlight the significant reduction in office SBP in RDN vs sham patients amongst non-African American subjects in Symplicity HTN-3 (-15.2 vs -8.6 mmHg, p=0.01) (Kandzari, Bhatt et al. 2015). In fact, African American and non-African American subjects had similar office SBP responses 6 months after RDN (-15.5 and -15.2 mmHg respectively), and the difference in the office SBP outcomes lies in the sham arm of the study (Kandzari, Bhatt et al. 2015). Amongst the sham group, African American participants demonstrated a borderline significant greater reduction in office SBP than non-African American subjects (-17.8 vs -8.6 mmHg, p=0.057)(Flack, Bhatt et al. 2015, Kandzari, Bhatt et al. 2015). Flack et al.'s recent multivariate analysis of Symplicity HTN-3 demonstrated that African American race did not independently predict SBP outcomes in either the RDN or sham groups, however, in the sham group the interaction between African American race and being prescribed at least one antihypertensive medication three times per day was associated with a greater reduction in office SBP at 6 months (Flack, Bhatt et al. 2015). In the sham group there was also a trend towards a greater reduction in office SBP for patients living in the south/south-eastern regions of the USA (Flack, Bhatt et al. 2015); areas which have previous been associated with lower rates of medication adherence (Couto, Panchal et al. 2014).

In Symplicity HTN-3 African American participants were taking a greater number of antihypertensive medications and had more complex medication regimes than non-African Americans (Flack, Bhatt et al. 2015). Individuals with complex drug regimens or who are prescribed a greater number of medications may be particularly likely to be non-adherent, and hence more vulnerable to a Hawthorne effect if enrolled in a clinical

trial (Baggarly, Kemp et al. 2014, Marquez-Contreras, Gil-Guillen et al. 2014). Hameed et al. addressed this issue by using directly observed medication administration with subsequent BP monitoring to confirm adherence prior to RDN (Hameed, Pucci et al. 2015). Their cohort of 34 patients achieved a response rate of 51% with an office BP reduction of -15/-6 mmHg (p=0.01/0.2) at 6 months, which is unlikely attributable to improved medication adherence. In a small Norwegian study, 5/18 patients were excluded from RDN following ABPM assessment after witnessed medication intake as part of the screening process (Fadl Elmula, Hoffmann et al. 2013). Given that at least 50% of patients with treatment resistant hypertension are known to be non-adherent with their medications (Jung, Gechter et al. 2013), more thorough assessment of medication adherence at screening, and during follow-up, should be mandatory in order to assess true drug resistance and establish any unreported changes in medication. The SYMPATHY trial, published this year, used blinded liquid chromatography analysis to evaluate adherence in 95 patients undergoing RDN and 44 controls; 80% of patients were not fully adherent with medication, and in this study, RDN was not superior to usual care as a treatment for resistant hypertension (de Jager, de Beus et al. 2017)

An alternative approach to the problem of medication adherence is to assess the effect of RDN on patients who are not taking any BP medications. Data have been published for 53 patients from 8 centres who received RDN whilst off medication (De Jager, Sanders et al. 2016). There was a reduction in BP in this cohort post-RDN, with a mean change in 24-h SBP of -5.7 mmHg (p = 0.04) and a mean change in office SBP of -13.1 mmHg (p = 0.001). The full results of the SPYRAL HTN ON-MED & OFF-MED studies (Medtronic (Kandzari, Kario et al. 2016)), and similarly REDUCE HTN: REINFORCE (V2, Vessix) and RADIANCE-HTN SOLO &TRIO (Paradise, ReCor Medical) (see Table 2-3) will hopefully provide a more robust evidence base for the efficacy of RDN in lowering BP in resistant hypertension.

1.1.1.1.3 Procedure: RDN Technique

One of the main critiques of Symplicity HTN-3 has been inadequate denervation due to operator inexperience/inadequate proctoring; there were 111 operators across 88 sites, of whom 31% contributed only 1 procedure and 23% contributed ≥5 procedures (Bhatt, Kandzari et al. 2014). This contrasts with the greater BP reductions seen in the Global Symplicity Registry in which 59% of operators performed >15 procedures (Bohm, Mahfoud et al. 2015). Only 19/364 patients received per-protocol RDN in Symplicity HTN-3 and this, along with the confounding effects due to medication changes in 39% of the population, renders the trial difficult to interpret (Bhatt, Kandzari et al. 2014, Kandzari, Bhatt et al. 2015).

It is possible that patients who only receive partial renal denervation may have an increase in BP due to unopposed action of the (usually inhibitory) reno-renal reflexes (Protasoni, Golin et al. 1996). Alternatively, partial denervation could cause sensitisation of those nerves that remain, inflammation of the nerves, or growth of new nerves which could exacerbate the degree of hypertension (Booth, Nishi et al. 2015, Sakakura, Tunev et al. 2015).

So how much denervation is required? In Symplicity HTN-1 a subset of patients underwent assessment with norepinephrine spillover, a validated technique for assessing regional sympathetic tone (Meredith, Esler et al. 1991); a 47% reduction in sympathetic nerve activity appeared sufficient to achieve a reduction in BP (Krum, Schlaich et al. 2009, Esler 2014). Further analyses by Esler et al. have shown that denervation following renal nerve ablation is highly variable between individuals and it is clear that the procedure is far more technically challenging than previously considered (Esler 2014, Tzafriri, Keating et al. 2015).

When the Symplicity catheter was first launched, operators were advised to prioritise ablation of the proximal superior aspect of the renal artery in order to target the highest density of renal nerves. However, review of novel anatomical human data indicates that the renal nerves accessible to intraluminal RF energy lie more distally in the renal artery adventitia (Sakakura, Ladich et al. 2014). Indeed, there is evidence that the right renal artery is more densely innervated than the left renal artery, and that the anterior and superior quadrants have more innervation than the posterior and inferior quadrants, along with a greater density of innervation in the distal versus proximal renal artery (Imnadze, Balzer et al. 2016). There are some data to counter this argument; Chen et al. did not see a benefit of full length renal artery denervation when compared with limited proximal ablation (Chen, Ling et al. 2016). However, based on a porcine model, Mahfoud at al. suggest that the denervation strategy should be device specific, with a distal main renal artery ablation strategy when using the EnligHTN catheter, and a distal main plus branch renal artery strategy when using the Symplicity Spyral catheter, although it is of note that all of the strategies used in this study resulted in a similar reduction in renal noradrenaline concentration (Mahfoud, Pipenhagen et al. 2017). Lesion placement (distal plus branch ablation with the Symplicity Spyral catheter), rather than the number of ablation per se, has been shown to correlate with a reduction in renal noradrenaline in pigs (Mahfoud, Tunev et al. 2015), and a greater reduction in mean 24-hour ABPM when compared with the conventional main renal artery ablation approach in humans (Petrov, Tasheva et al. 2018). In light of these novel data, it seems probable that operators following the earlier guidance may have been targeting the wrong part of the artery, resulting in inadequate denervation (Mahfoud, Edelman et al. 2014).

The impact and management of accessory renal arteries in patients undergoing RDN has also generated conflicting findings. Accessory renal arteries are identified in around 30% of those with resistant hypertension (Ewen, Ukena et al. 2016, Burchell, Rodrigues et al. 2017), although one group has reported accessory renal arteries in 59% of hypertensives versus 32% in normotensive controls (VonAchen, Hamann et al. 2016). It has been reported that the presence, and non-treatment, of accessory renal arteries is associated with a lack of response to RDN (VonAchen, Hamann et al. 2016), but this finding is not consistent across all studies (Ewen, Ukena et al. 2016).

If the 'completeness' of denervation relates to procedural success, then a method for assessing the degree of renal nerve disruption achieved would be of significant clinical benefit and guide development of evolving catheter technologies. Techniques including direct electrical renal nerve stimulation, urinalysis for breakdown products of renal sympathetic nerve degradation (e.g. tyrosine hydroxylase) and measurement of reflex responses to afferent renal nerve stimulation with agents such as adenosine or bradykinin are under evaluation and are reviewed in more detail in Section 5.5 (Katholi,

Whitlow et al. 1984, Esler 2015, Gal, de Jong et al. 2015). Developing a test which can be used to assess for successful renal denervation at the time of the procedure, is another major aim of this thesis.

2.3.4 Renal denervation after Symplicity HTN-3

2.3.4.1 Study design

Howard et al. provide a good summary of three biases which can lead to the overestimation of the antihypertensive effect of RDN in clinical trials (Howard, Shun-Shin et al. 2016).

1. Regression to the mean. The majority of RDN studies have selected patients with severe hypertension (office SBP >160 mmHg). Blood pressure varies over time, and patients may have been preferentially selected for the study when their BP was higher than their long-term average, with subsequent regression to the mean during unselected long-term follow-up. This may be addressed by adding a randomized control group with similar inclusion criteria to ensure that regression to the mean occurs similarly in both arms of the study.
2. Asymmetrical data handling. If a physician records BP following an intervention but fails to see the fall in BP that they expect, then they are more likely to repeat the measure rather than document a seemingly incorrect value. This can be addressed using studies in which the physician is blinded during data collection.
3. Confounding. This describes a blood pressure reduction following RDN due to factors other than the denervation and would include changes in medication adherence and any placebo affect from the procedure. It could be addressed by blinding the patient using a sham procedure to counter any placebo affect from the intervention, and by ensuring that any changes in medication adherence due to enrollment in the study are equally present in both sham and treatment arms of the study. Clearly improved measures to standardise medication regimes and confirm stable medication adherence would also help to confirm robust outcome measures.

Several meta-analyses have been published following Symplicity HTN-3 which put the results of this study into a wider context. Most recently the European Network COordinating research on Renal Denervation (ENCOReD) have published a meta-analysis of 10 RCTs which shows no significant effect of renal denervation on BP in resistant hypertension (Fadl Elmula, Feng et al. 2017). Likewise, Krakoff et al. reviewed findings from nearly 2000 patients treated with RDN from meta-analysis and registry data and concluded that RDN was not superior to standard medical management of hypertension (Krakoff and Sartori 2016). In contrast, Zhang et al. found RDN to be superior to pharmacotherapy in their analysis of11 controlled and randomised-controlled trials (Zhang, Wu et al. 2016). Interestingly, Qi et al. demonstrated that RDN efficacy differed between unblinded and blinded trials, with no significant effect on BP seen in the blinded studies (Qi, Cheng et al. 2016). In another meta-analysis, RDN was reported to be effective at lowering BP, but on closer consideration, this beneficial effect on BP was not seen when only RCTs were included in the analysis (Sun, Li et al. 2016).

Data from the ENCOReD group emphasises the importance of using ABPM outcomes but did not necessitate sham procedures in RDN studies (Fadl Elmula, Feng et al. 2017). In a study focussing on causes of bias, Howard et al. found that (i) office BP reductions were much larger than ABPM reductions following RDN, but only in unblinded trials, (ii) the unblinded study design was associated with a greater office BP reduction in the treatment arm versus the control arm, and (iii) that adding a randomised control arm did not reduce bias unless it was blinded. Study design is clearly extremely important for all future trials of RDN.

In the wake of Symplicity HTN-3, Medtronic launched the SPYRAL HTN Global Clinical Trial Program of renal denervation in the absence (SPYRAL HTN OFF-MED) and presence (SPYRAL HTN ON-MED) of antihypertensive medications (Kandzari, Kario et al. 2016). These studies aim to address many of the criticisms raised about the early Symplicity studies:

1. Patients would be studied whilst on a standardised three-drug antihypertensive regime (ON-MED) or off all antihypertensive medication (OFF-MED), with adherence to (or abstinence from) medication confirmed by liquid chromatography–tandem mass spectroscopy.
2. The studies would enroll patients with moderate, combined systolic and diastolic, hypertension with a 24hr ambulatory systolic blood pressure of ≥140 mmHg and <170 mmHg and an office diastolic BP of ≥90 mmHg
3. The primary outcome measure would use mean 24hr ABPM results, with monitoring following in-office-observed medication in the ON-MED group.
4. The procedure would be performed using the Symplicity Spyral catheter to try to ensure four-quadrant ablation, and a main, branch and accessory renal artery ablation strategy was advocated, with one experienced operator per site.
5. Patients and clinicians undertaking hypertension management would be blinded to the study arm (treatment versus sham) for one year.

The use of 24hr ABPM data as an outcome measure in these studies, may also prove to better reflect the regression of end organ damage in these significantly hypertensive patients since nocturnal hypertension in particular correlates strongly with cardiovascular morbidity and mortality (Mancia, Zanchetti et al. 1997, Hermida, Ayala et al. 2014). The ASCOT study found that nocturnal SBP was superior when compared with office SBP in predicting stroke (Dolan, Stanton et al. 2009), and in the Dublin Outcome study nocturnal BP was an independent risk factor for cardiovascular mortality, with a 10 mmHg increase in nocturnal SBP conferring a 21% increased risk of cardiovascular mortality (Dolan, Stanton et al. 2005). Mahfoud et al. reported reductions in both daytime and night-time SBP, but no improvement in nocturnal BP dipping status, following RDN (Mahfoud, Ukena et al. 2013). In Symplicity HTN-3, 21% of patients treated with RDN converted to become BP dippers following treatment, however, this did not differ from the 15% increase in dipper status in the sham cohort (p=0.30) (Bakris, Townsend et al. 2014). Ultimately BP is only a surrogate marker for the physical and economic burden inflicted by conditions such as stroke, myocardial infarction and chronic kidney disease (Metoki, Ohkubo et al. 2006, Mahfoud, Ukena et al. 2013).

2.3.4.2 Morbidity and Mortality Outcomes

The predominant outcome measure in published RDN studies is BP reduction, but does a fall in BP equate to an improvement in the morbidity and mortality associated with chronic hypertension? RDN has been shown to result in regressions in left ventricular hypertrophy (LVH) and atrial enlargement, and to improve cardiac function in patients with evidence of hypertensive heart disease (Mahfoud, Urban et al. 2014, Tsioufis, Papademetriou et al. 2015, Lu, Wang et al. 2016, Tsioufis, Papademetriou et al. 2016, Delacroix, Chokka et al. 2018, Kordalis, Tsiachris et al. 2018, Wang, Yang et al. 2018). RDN also reduces arterial stiffness, with a reduction in pulse wave velocity greater than that expected secondary to a reduction in BP alone, suggesting an additional effect due to sympathoinhibition; a process which may also have important prognostic implications (Baroni, Nava et al. 2015, Delacroix, Chokka et al. 2018). In contrast, Verloop et al. report no improvement in target organ damage despite a modest reduction in BP 12 months post denervation (Verloop, Vink et al. 2015).

Attempts have also been made to assess any improvement in quality of life following RDN; whilst patients with chronic resistant hypertension have a subjective reduction in quality of life and RDN can improve some of these indices, the degree of BP reduction does not correlate with these subjective improvements (Lambert, Hering et al. 2012). Twelve months post-RDN, patents with resistant hypertension have shown some improvement in the mental health related aspects of quality of life (Lambert, Hering et al. 2015), with data from the Global Symplicity Registry showing a particular improvement in anxiety and depression (Kindermann, Wedegartner et al. 2017).

Studies completed thus far have not been of sufficient power or duration to address the effect of RDN on major cardiovascular events (MACE) or mortality, however, the proposed EnligHTNment trial (St Jude) was due to be the first large-scale study to examine whether RDN also reduces the risk of major cardiovascular events such as heart attack, stroke and death in patients with resistant hypertension (2017), however this has not come into fruition. Based on the 32 mmHg reduction in SBP reported in Symplicity HTN-2, RDN was estimated to reduce the 10 year and lifetime relative risk of (10-year/lifetime relative risks) stroke (0.70/0.83), myocardial infarction (0.68/0.85), heart failure (0.79/0.92) and end-stage renal disease (0.72/0.81), and to give a cost-saving of $31,460 per quality-adjusted life-year (Geisler, Egan et al. 2012). These estimates will need to be revised in the light of more recent (and pending) outcome data, and it is likely that studies powered to assess the impact of RDN on MACE or mortality will not be undertaken unless a BP lowering effect following RDN has been demonstrated more conclusively by the ongoing Symplicity Spyral HTN studies.

In the UK a joint societies consensus statement currently advises that RDN should not be used in routine clinical practice but does support the treatment of patients as part of ongoing clinical trials using the technique (Lobo, de Belder et al. 2015).

2.3.5 Spyralling forward

The preliminary outcome data for the SPYRAL HTN-ON and -OFF MED studies have now been published (Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018).

Three-month outcome data for the first 80 patients recruited into the SPYRAL HTN-OFF MED study reported significant reductions both ambulatory and office BP in the RDN group (n=38, 24-hr SBP -5·5 mmHg (95% CI -9·1 to -2·0; p=0·003), 24-hr DBP -4·8 mmHg (-7·0 to -2·6; p<0·0001), office SBP -10·0 mmHg (-15·1 to -4·9; p=0·0004), and office DBP -5·3 mmHg (-7·8 to -2·7; p=0·0002)), not seen in the sham-control group (n=42, 24-hr SBP -0·5 mmHg (95% CI -3·9 to 2·9; p=0·76), 24-hr DBP -0·4 mmHg (-2·2 to 1·4; p=0·64), office SBP -2·3 mmHg (-6·1 to 1·6; p=0·24), and office DBP -0·3 mm Hg (-2·9 to 2·2; p=0·81)) (Townsend, Mahfoud et al. 2017). Notably, subjects received an average of 43.8 ablations with treatment applied to both main and branch renal arteries, which was significantly higher than in earlier studies (e.g. mean of 11.2 ablations in Symplicity HTN-3 (Bhatt, Kandzari et al. 2014)), and overall compliance with the requirement to be off all antihypertensive medication was 85.5% (Townsend, Mahfoud et al. 2017).

In the SPYRAL HTN-ON MED study, data were required out to 6-months post-RDN before a significant difference in BP outcome was observed between those treated with RDN (n=38) versus a sham procedure (n=42) (Kandzari, Bohm et al. 2018). There was a significant reduction in both ambulatory and office BP at 6 months in the RDN treatment group (24-hr SBP -7·0 mmHg (95% CI -12·0 to -2·1; p=0·006), 24-hr DBP -4·3 mmHg (-7·8 to -0·8; p=0.02), office SBP -6·6 mmHg (-12·4 to -0·9; p=0·03), and office DBP -4·2 mmHg (-7·7 to -0·7; p=0·02) (Kandzari, Bohm et al. 2018). As with the OFF-MED study, patients underwent intensive main and branch renal artery ablations (mean 45.9 ablations), and despite the standardised medication regime, adherence was only around 60% over the course of the study (Kandzari, Bohm et al. 2018).

The third key study supporting a clinical BP lowering effect for RDN published within the last year is the RADIANCE HTN-SOLO trial (Azizi, Schmieder et al. 2018). This sham-RCT investigated the antihypertensive effect of RDN using an endovascular ultrasound ablation system with patients off medication for 4 weeks prior, and 2 months after, denervation. There were significant differences in the reductions in both 24-hr ambulatory and office BP between the RDN (n=74) and sham groups (n=72; 24hr SBP; -8.5 ± 9.3 mmHg vs -2.2 ± 10.0 mmHg, p=0.0001, 24hr DBP; -5.1 ± 5.9 mmHg vs -2.6 ± 6.5 mmHg, p=0.01, office SBP; -10.8 ± 13.6 mmHg vs -3.9 ± 17.4 mmHg, p=0.007, office DBP; -5.5 ± 8.4 mmHg vs -1.2 ± 10.0 mmHg, p=0.005, RDN vs sham, respectively (data mean ± SD)) (Azizi, Schmieder et al. 2018). Interestingly, a mean of only 5.4 ultrasound emissions was required to achieve a greater reduction in SBP than that observed in the SPYRAL studies (Azizi, Schmieder et al. 2018).

These positive BP outcomes seen in more rigorously designed trials have reignited interest in RDN, but as the individual data show, the response to RDN is still highly variable (see Figure 2-2-13), even with more intensive ablation strategies, and adherence was difficult to standardise even with a more structured protocol. The questions posed in this pilot study are therefore as relevant as ever.

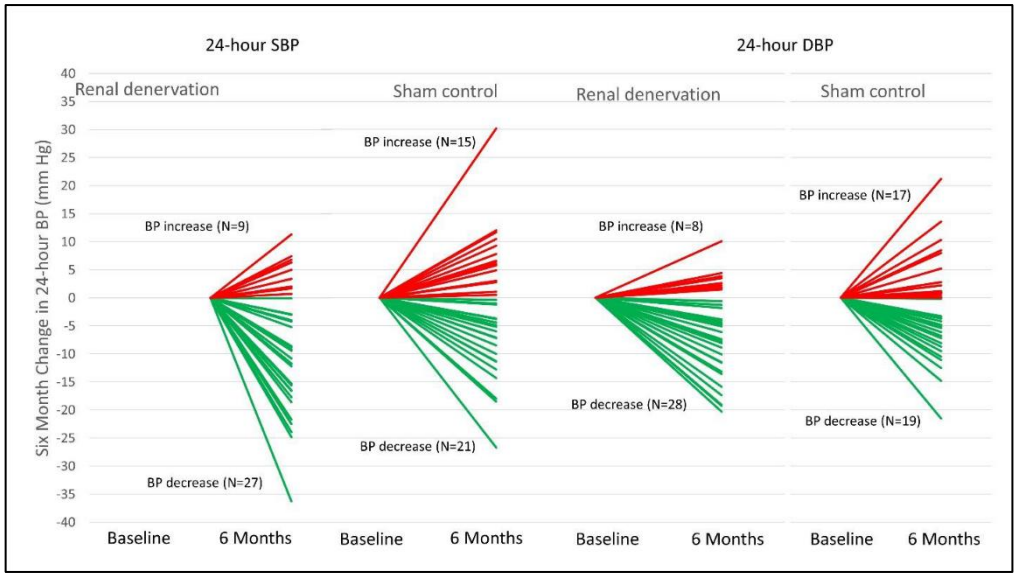


Figure 2-2-13. Changes in 24-hour blood pressure at 6 months after denervation for individual patients in the SPYRAL HTN-ON MED study.
Red lines show an increase in 24-hr BP following RDN and green lines indicate a decrease in BP following RDN. (Kandzari, Bohm et al. 2018)

2.3.6 Renal denervation: procedural safety

The data available to date suggest that RDN has an acceptable safety profile (Krum, Schlaich et al. 2009, Bhatt, Kandzari et al. 2014, Esler, Bohm et al. 2014, Bohm, Mahfoud et al. 2015). The Symplicity HTN-1 Investigators report 4 acute complications in their expanded cohort of 153 patients (one renal artery dissection and 3 femoral pseudoaneurysms/haematomas), none of which resulted in any long-term adverse effects (2011). Renal angiographic studies identified focal renal artery irregularities immediately after radiofrequency energy delivery, none of which was judged as flow limiting at procedure termination (Krum, Schlaich et al. 2009). In 81 patients with repeat renal artery imaging at 6 months there were no cases of novel renal artery stenosis (RAS), but one patient developed progression of a pre-existing stenosis which was successfully stented (2011). In Symplicity HTN-2 there was a further renal artery dissection requiring stenting, and one patient had to be re-admitted following a hypotensive episode (Esler, Krum et al. 2012).In Symplicity HTN-3, there was no difference in major adverse events between the RDN and sham groups; in the RDN group (n=361) there were two deaths (cause unspecified), 5 patients had an increase in creatinine of >50% from baseline, 1 patient had a vascular complication requiring treatment and one patient developed a de novo renal artery stenosis of >70%, no renal artery interventions were required (Bhatt, Kandzari et al. 2014). There were no major procedural or safety adverse events reported in either of the SPYRAL HTN studies (Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018).

Data from the Global Symplicity registry (n=998) also support the procedural safety of RDN using the Symplicity catheter (Bohm, Mahfoud et al. 2015). Acutely, there were 2 (0.2%) renal artery interventions after dissection, 3 pseudoaneurysms (0.3%), and 1 haematoma (0.1%), and during the first 6 months after the procedure, there was 1 new

RAS >70%, 3 cases of an increase in creatinine of >50%, and 5 cases of hospitalisation for a hypertensive emergency. The meta-analysis from the ENCOReD group reported no adverse effect of RDN on renal function (Fadl Elmula, Jin et al. 2015). Repeat imaging of the renal arteries at least 6 months after radiofrequency denervation using either optical coherence tomography and angiography, or MRI angiography, support the long-term safety profile of the procedure from a vascular perspective (Roleder, Skowerski et al. 2016, Schmid, Schmieder et al. 2016).

The safety profiles for the other commercially available denervation catheters and therapeutic modalities are similarly positive. In EnligHTN-I (n=45), there were three serious adverse events including progression of existing hypertensive renal disease, hypotension, and the progression of a pre-existing RAS, and some transient minor adverse events including non-flow limiting vasospasms, vascular access site haematomas, vasovagal episodes, bradycardia, transient haematuria, pain, and nausea (Worthley, Tsioufis et al. 2013). REDUCE-HTN (n=146) using the Vessix V2 catheter reported one mild procedural vessel dissection which did not require intervention, along with two access-site infections, one pseudoaneurysm at the access site, and one femoral artery thrombus which all resolved (Sievert, Schofer et al. 2015). During the first 6 months of follow-up, four patients in the REDUCE-HTN study had progression of existing renal artery stenoses, one patient had a hypertensive emergency requiring hospitalisation and fifteen patients (11%) had an eGFR reduction >25%, although overall, mean eGFR remained unchanged (Sievert, Schofer et al. 2015). Use of the Paradise ultrasonic catheter was associated with transient vasospasm, but no long term sequelae or evidence of new RAS on follow-up imaging (n=50) (Fengler, Hollriegel et al. 2017), further supported by recent data from the RADIANCE HTN-SOLO which reported no major adverse events (Azizi, Schmieder et al. 2018). The Kona Medical Surround Sound system was most frequently associated with post procedural back pain in 32 of 69 subjects, but there were no short or long-term effects on renal function or evidence of renal vascular or parenchymal damage (Neuzil, Ormiston et al. 2016).

There is also now data from care reports/case series, to support the safety and efficacy of a second, redo, renal denervation procedure in patients who fail to respond to initial RDN therapy, or who have an increase in BP after primary treatment success (Lambert, Nahler et al. 2013, Prochnau, Heymel et al. 2014, Daemen, Feyz et al. 2017).

These data demonstrate an acceptable safety profile for the use of RDN in the treatment of resistant hypertension, and whilst RDN has been used safely in patients with an eGFR of <45 ml/min per 1.73 m^2 (Hering, Mahfoud et al. 2012), caution should be taken when treating patients with pre-existing renal artery stenoses or chronic kidney disease, and these patients should be consented accordingly.

2.3.7 Renal denervation for indications other than hypertension

If RDN results in a reduction in sympathetic nerve activity (SNA), then there is a potential benefit of renal denervation in other states of sympathetic activation such as chronic heart failure, sleep apnoea, polycystic ovarian syndrome, insulin resistance, chronic renal failure and cardiac arrhythmias (Schlaich, Straznicky et al. 2011, Hering, Esler et al. 2012, Hering, Mahfoud et al. 2012, Schlaich, Hering et al. 2012, Wilson, Kistler et al.

2014, Kario, Bhatt et al. 2016). These conditions interact and frequently co-exist in individual patients, furthermore, successful renal nerve ablation for the treatment of hypertension may also prevent development of these associated pathologies. Since these diseases are a major drain on NHS resource, RDN may become a highly cost-effective procedure if shown to be effective and applied to the appropriate patient population.

2.3.7.1 Heart failure

Heart failure is associated with chemohypersensitivity and sympathoexcitation (Esler and Kaye 2000, Ding, Li et al. 2011, Paton, Sobotka et al. 2013, Bohm, Ewen et al. 2017). I have previously reviewed the role of chemohypersensitivity in modulating venous capacitance through activation of the sympathetic nervous system in heart failure (Burchell, Sobotka et al. 2013). The REACH-HF trial was the first study to investigate the use of RDN in systolic heart failure, the study showed no adverse effect on BP, with an improvement in 6-minute walk distances and patient symptoms (n=7) (Davies, Manisty et al. 2013). RDN may be able to improve symptoms due to congestion in CHF, without reducing BP, through redistribution of blood flow due to a reduction of sympathetic activation, which modulates control of the venous reservoir and sodium water retention (Bohm, Ewen et al. 2017).

The results of the SYMPATHY-HF feasibility study have recently been published (Hopper, Gronda et al. 2017). RDN was performed in 39 patients with a left ventricular ejection fraction (LVEF) of <40 %; there was a significant reduction in NT-proBNP (N-terminal pro-brain natriuretic peptide) following the procedure, but no change in LVEF. In a meta-analysis of two controlled (80 patients) and 2 uncontrolled (21 patients) studies of patients with heart failure and reduced LVEF, Fukuta et al. report that 6 months after RDN, there was a greater increase in EF and a greater decrease in LV end-diastolic diameter in patients who had undergone RDN versus controls (Fukuta, Goto et al. 2017). Gao et al. reported improvements in NT-proBNP, LVEF and NYHA (New York Heart Association) class in patients with chronic heart failure following RDN (Gao, Yang et al. 2018), whereas Geng et al. report that those with early onset heart failure are more likely to benefit from RDN then those with late-stage disease (Geng, Chen et al. 2018). These results demonstrate that RDN is safe and feasible in the heart failure population, however, there are potential issues regarding bias as seen in the studies for resistant hypertension and further research is required to support the use of RDN in this condition.

2.3.7.2 Sleep apnoea

In obstructive sleep apnoea (OSA), intermittent hypoxia, microarousal, chemoreceptor activation, decreased pulmonary stretch receptor activation, and increased negative thoracic pressure, are associated with sympathoexcitation, insulin resistance and pro-inflammatory effects (Kario 2009). OSA is not just a cause of excess sympathetic tone, but also a consequence of sympathoexcitation. A decrease in SNA following RDN has been hypothesised improve OSA by reducing fluid retention, decreasing peri-pharyngeal fluid accumulation and pharyngeal wall thickness, and/or by resetting the dysrhythmicity of pharyngeal muscles (Shantha and Pancholy 2015). The impact of renal

denervation on the severity of OSA in patients with resistant hypertension has shown inconsistent results. Some studies have shown an improvement in apnoea-hypopnoea index (Witkowski, Prejbisz et al. 2011, Shantha and Pancholy 2015), whilst other studies report no improvement in OSA severity following RDN (Daniels, De Freitas et al. 2017). Data from the Symplicity HTN-3 study suggests that patients with OSA may be particularly responsive to RDN with regard to BP reduction and an improvement in nocturnal BP dipping status (Kario, Bhatt et al. 2016). In contrast, there was no difference in the BP response to RDN between patients with and without self-reported OSA in the Global Symplicity Registry (Linz, Mancia et al. 2017).

2.3.7.3 Metabolic conditions

According to the statement of the American Heart Association, metabolic syndrome is defined as the presence of ≥3 of the following 5 features: abdominal obesity, hyperglycaemia, hypertension, hypertriglyceridemia, and low high-density lipoprotein cholesterol levels (Alberti, Eckel et al. 2009). The syndrome is associated with a 2-fold risk of cardiovascular disease (Alberti, Eckel et al. 2009).

In 2011, Mahfoud et al. reported improvements in glucose metabolism (response to an oral glucose tolerance test (OGTT)) and insulin resistance (homeostasis model assessment – insulin resistance (HOMA-IR) following RDN in patients with resistant hypertension (Mahfoud, Schlaich et al. 2011). Since this publication there has been conflicting evidence surrounding the effect of RDN on metabolic syndrome. Data from the Polish registry (RDN-POL) suggest an improvement in glucose metabolism following RDN, with a reduction in 2-hour glucose following an OGTT amongst ABPM responders (Kadziela, Prejbisz et al. 2016). In patients with metabolic syndrome and hypertension, renal denervation has been shown reduce MSNA and restore the normal neural response to oral glucose loading as compared to controls, although HOMA-IR was not affected (Tsioufis, Dimitriadis et al. 2017). Insulin resistance also failed to improve following RDN in 23 patients assessed using a rigorous hyperinsulinemic-euglycemic step clamp technique (Miroslawska, Gjessing et al. 2016). The Denervation of the Renal Arteries in Metabolic Syndrome (DREAMS) study aimed to investigate the effect of RDN on insulin sensitivity and BP in patients with metabolic syndrome; the study of 29 patients showed a moderate decrease in 24hr ABPM, but no change in MSNA or insulin sensitivity (assessed by an oral glucose tolerance test (OGTT) out to 12 months after the procedure (Verloop, Spiering et al. 2015).

In patients with hypertension, skeletal muscle tissue shows several features that would predispose to insulin resistance, including, lower blood flow and delivery of insulin and glucose to skeletal muscle tissue because of vasoconstriction and vascular hypertrophy, fewer slow-twitch insulin-sensitive muscle fibres with increased fat distributed between the skeletal muscle fibres, and abnormal metabolic signalling responses to insulin (Rojas, Velasco et al. 2012). The mechanisms through which RDN may improve glucose metabolism are unknown, but potential factors may include a decrease in MSNA (with a decrease in vascular α-adrenergic tone, leading to an improved distribution of skeletal muscle blood flow), decreased RAAS activity, enhanced sensitivity to insulin's non-esterified fatty acid–lowering actions, decreased gluconeogenesis, and changes in glucose transporters and glucagon secretion (Egan 2011).

Polycystic ovarian syndrome (PCOS) is another condition characterised by metabolic disturbance and sympathoexcitation. Data from two subjects with PCOS, obesity and hypertension showed a decreased in SNA, BP and insulin resistance, and there was even a return of menstruation in one patient after an amenorrhoeic period of three years, following RDN (Schlaich, Straznicky et al. 2011).

2.3.7.4 Chronic kidney disease

Chronic kidney disease (CKD) is associated with sympathoexcitation and can be both a cause and effect of hypertension (Schlaich, Socratous et al. 2009). The first question when considering RDN in the context of CKD is that of safety. Hering et al. reported no adverse effect on renal function in a small study of 15 patients with moderate-severe CKD (eGFR <45 ml/min/1.73m^2), with improvements seen in office BP, nocturnal BP dipping status and augmentation index (Hering, Mahfoud et al. 2012). Schlaich et al. report favourable safety data for the use of RDN in dialysis patients with end-stage renal disease, although RDN could not be completed in 2/12 patients due to renal artery atrophy (Schlaich, Bart et al. 2013).

Moving forward, there are data to suggest that RDN may result in an improvement in renal function in patients with CKD (Sata and Schlaich 2016). In a cohort of 30 patients with mild-moderate CKD, Kuichi et al. have reported significant improvements in ABPM (152±17/93±11 vs 132±14/84±12 mmHg), eGFR (61.9±23.9 vs 88.0±39.8 mL/min/1.73 m^2), and urine albumin:creatinine ratio (99.8 vs 11.0 mg/g (all p<0.0001)) (Kiuchi, Graciano et al. 2016). Ott et al. observed renal function in 27 patients with stage 3 and 4 CKD for 3 years before, and 1 year after, RDN: prior to RDN, eGFR declined by -4.8 ± 3.8 ml/min/1.73 m^2 per year, and after RDN eGFR improved by +1.5 ± 10 ml/min/1.73 m^2 at 12 months (P = 0.009) (Ott, Mahfoud et al. 2015). In the latter study, the change in eGFR after denervation did not correlate with the reduction in mean 24hr ABPM, and whilst this may reflect the small sample size, it may also indicate that the improvement in eGFR is not simply due to a reduction in BP but may also reflect changes in renal sympathetic tone and/or sympathetically mediated inflammation.

2.3.7.5 Cardiac arrhythmias

Renal denervation has also been used as an adjunct in the treatment of cardiac arrhythmias, working on the theory that RDN can reduce proarrhythmic sympathetic drive. In hypertension, cardiac remodelling with atrial enlargement and autonomic sympathovagal imbalance, increase the propensity for atrial fibrillation (AF) (McArdle, deGoma et al. 2016). Sympathetic activity increases Ca^{2+} influx and Ca^{2+} release from the sarcoplasmic reticulum, thus enhancing automaticity and triggered activity in the atria (Nammas, Airaksinen et al. 2016). The myocardium is vulnerable to increased SNA following myocardial infarction due to increased sensitivity to circulating catecholamines, up-regulation of beta-adrenergic receptors and nerve sprouting along the border zones of infarcts (Bradfield, Vaseghi et al. 2014). The mechanisms for a reduction in ventricular arrhythmias following RDN is likely to be multifactorial because of the wide range of underlying pathologies (e.g. acute ischaemia, post-infarct scar tissue, dilated or hypertensive cardiomyopathy), but may include, improved volume

status in heart failure patients, decreased left ventricular hypertrophy, a decrease in arrhythmias triggered through the sympathetic effect on myocardial Ca^{2+} signaling, and decreased repolarisation heterogeneity at scar border zones (Bradfield, Vaseghi et al. 2014).

In a first in man study, Pokushalov et al. compared pulmonary vein isolation (PVI; n=14) against PVI plus RDN (n=13) in patients with drug resistant hypertension and symptomatic paroxysmal or persistent AF refractory to ≥2 antiarrhythmic drugs: 69% of those treated with PVI + RDN, versus 29% of those treated with PVI alone, were AF free at 12 months (Pokushalov, Romanov et al. 2012). This study was criticised for its small sample size and the limited capacity for 24hr Holter monitoring to detect asymptomatic tachyarrhythmias (Nammas, Airaksinen et al. 2016). The patients treated with RDN also had a significant reduction in office BP, not seen in the PVI-only group, and it is therefore not clear whether the reduced arrhythmia burden was due to a reduction in BP or reduced cardiac sympathoexcitation. To address some of these issues, the same group have published data on patients with paroxysmal and/or persistent AF and resistant hypertension, who underwent PVI-only (n=37) or PVI+RDN (n=39), and implantable cardiac monitor implantation (Romanov, Pokushalov et al. 2017). RDN was associated with a reduction in BP, AF recurrence and AF burden, with a significant correlation between the BP reduction and the decline in AF burden; a BP reduction of 5-10 mmHg was accompanied by a 7.0% decreased AF burden, with greater BP reduction (up to 20 mmHg) associated with a 17.7% lower AF burden (Romanov, Pokushalov et al. 2017). There is also evidence to show that RDN may improve the efficacy of PVI in patients with CKD (Kiuchi, Chen et al. 2017), and that the intervention could improve ventricular rate control in patients with persistent AF and hypertension (Qiu, Shan et al. 2016). Sleep disordered breathing, including OSA, is present in 40-50% of those with AF (Linz, Linz et al. 2016), and in a porcine model of OSA, RDN reduced spontaneous AF and post-apnoeic BP rises (Linz, Hohl et al. 2013).

Renal denervation may also have a role in the treatment of ventricular tachyarrhythmias (VT) through a reduction in SNA. This is an intuitive step, since blockade of the β-adrenergic receptors is used routinely in clinical practice to prevent VT (McArdle, deGoma et al. 2016). In a porcine, post-myocardial infarction model, there was a 100% reduction in the rate of spontaneous ventricular arrhythmias after RDN as compared with a 75% increase in the rate of spontaneous ventricular arrhythmias after a sham procedure, furthermore, in the infarcted myocardium, the presence of sympathetic nerves and level of neuropeptide-Y (a marker of SNA), were significantly lower in the RDN group (Jackson, Gizurarson et al. 2017). There have been multiple case reports and case series describing improvement in VT following RDN, including the adjunctive treatment of VT storm, polymorphic VT, and postinfarct VT, and VT in the context of dilated and hypertrophic cardiomyopathy and vasospastic angina (Ukena, Bauer et al. 2012, Hoffmann, Steven et al. 2013, Remo, Preminger et al. 2014, Scholz, Raake et al. 2015, Aksu and Guler 2017, Feyz, Wijchers et al. 2017).

Three small studies and limited registry data provide slightly more substantive evidence for the use of RDN in the treatment of VT. Armaganijan et al. presented a series of 10 patients with implantable cardioverter-defibrillators (ICDs), who underwent RDN for the treatment of refractory VT (Armaganijan, Staico et al. 2015). Interrogation of the ICDs over a 6-month period, showed a significant reduction in arrhythmia burden, with 8/10

responding with reduced levels of ventricular arrhythmia post-RDN. Similarly, Jiang et al. reported a reduction in ventricular arrhythmia burden in eight patients with ICDs in situ who were treated with RDN (Jiang, Zhou et al. 2018). Evranos et al. looked at the evidence for the use of RDN as an adjunct to cardiac VT catheter ablation (Evranos, Canpolat et al. 2016). Interrogation of the ICDs in these patients showed that those who had been treated with combined VT and renal nerve ablation had a substantial reduction in VT/ventricular fibrillation (VF) and antitachycardia pacing and shock therapies (both groups n=16). Finally, Ukena et al. have published data on the use of RDN in patients with chronic heart failure and refractory VT, from an international multicentre registry; there was a reduction in VT/ VF burden, but no reduction in BP or NYHA (New York Heart Association) classification following RDN, with 11/13 patients being free from VT/VF at 1 and 3 months after the procedure (Ukena, Mahfoud et al. 2016).

These preliminary data all suggest additional indications for RDN, moving beyond the treatment of resistant hypertension, to target other pathologies associated with sympathoexcitation. It is important, however, to exercise caution. These findings are based on case reports, case series, and at best small randomised controlled, but non-blinded, studies; many of the criticisms cited against Symplicity HTN-3 could be applied. Further research is required to establish the mechanism of action of RDN, and to confirm a sympathoinhibitory effect.

2.3.8 Other interventional strategies for the treatment of resistant hypertension

A review of RDN would not be complete without a summary of other, competing, interventional strategies for the treatment of drug resistant hypertension. These currently include baroreflex activation therapy (BAT), carotid body modulation, central iliac arteriovenous anastomosis (ROX procedure), carotid sinus stenting (Mobius device), vagal nerve stimulation, median nerve stimulation and deep brain stimulation.

2.3.8.1 Baroreflex activation therapy

Baroreflex sensitivity is known to be impaired in hypertension (see Section 2.1.2.3.2), and given this finding, baroreceptor stimulation presents a plausible therapeutic option for the treatment of drug resistant hypertension. The first-generation device, the Rheos System (CVRx, Inc., Minneapolis, Minnesota, USA), which consisted of large bilateral carotid sinus electrodes and a pulse generator (sited sub-clavicularly), was primarily limited by a high rate of serious adverse events, primarily including transient or permanent nerve damage and device infection, and the low battery life of the device (Illig, Levy et al. 2006, Scheffers, Kroon et al. 2010, Bisognano, Bakris et al. 2011, Hering, Schultz et al. 2016). The Rheos System did, however, show a stimulation dose related reduction in BP (Rheos Feasibility Trial, n=10, (Illig, Levy et al. 2006)), with a -21/-12 mmHg reduction in office SBP at 3 months (n=37) , sustained out to 2 years (-33/-22 mmHg, n=17) in the DEBuT-HTN (Device-Based Therapy of Hypertension) study (Scheffers, Kroon et al. 2010). The Rheos Pivotal Trial was a double-blind trial in which patients were implanted with the device (n=265) and then randomised to stimulation

on, or off, during the first six months, after which all devices were switched on (Bisognano, Bakris et al. 2011). Six months after implantation, the decrease in SBP was 16 ± 29 mmHg for those with the device on and 9 ± 29 mmHg for those with the device off. This endpoint did not reach significance (p = 0.08), although significantly more of those with the device on achieved an office SBP of <140 mmHg. Data compiled from all three studies using the first generation Rheos System have shown a sustained BP reduction out to six years post implantation (de Leeuw, Bisognano et al. 2017).

The second-generation device, the Barostim Neo (CVRx, Inc., Minneapolis, Minnesota, USA), has a much smaller unilateral electrode, with a substantially improved safety profile (Hoppe, Brandt et al. 2012). Furthermore, there is evidence to support a beneficial effect of BAT in patients who have failed to achieve BP control following RDN, with a 68% response rate to BAT in this cohort (n=28) (Wallbach, Halbach et al. 2016). BAT reduced proteinuria and albuminuria in 23 patients with CKD and resistant hypertension (Wallbach, Lehnig et al. 2014). BAT has also been trialled in patients with heart failure with reduced ejection fraction, with improvements in functional status, quality of life, exercise capacity and NT-proBNP (Bisognano, Bakris et al. 2011, Abraham, Zile et al. 2015).

Despite these positive findings, use of the Barostim Neo has experienced important limitations. In a study by Heusser et al., 12/18 patients with resistant hypertension treated with unilateral, unipolar BAT experienced stimulation related side-effects (including jaw or neck pain, globus or swallowing sensation, coughing, or voice problems), necessitating a reduction in the stimulus intensity which resulted in a reduced antihypertensive effect (Heusser, Tank et al. 2016). Stimulation at tolerable intensities reduced SBP by 16.9 ± 15.0 mmHg (p=0.002), but with considerable interindividual variability (range 0.0 to −40.8 mmHg). This BP reduction was more modest than the 26.0 ± 4.4 mmHg reduction in SBP seen in the Barostim Neo trial (Hoppe, Brandt et al. 2012), and this is attributed in part to insufficient carotid baroreceptor engagement due to issues with electrode placement and/or electrode design. The unipolar design of the device means that there is spread of the stimulus as it travels been the electrode and pacemaker box located in the upper chest, with the potential to cause side-effects through stimulation of the surrounding tissues. Furthermore, the small electrode must be positioned over a discrete sensitive area of the carotid sinus, which may vary anatomically between individuals, in order to achieve an optimal effect from this invasive procedure (Heusser, Tank et al. 2016).

Once again, mechanistic evidence, and evidence from RCTs is required to support the use of BAT in hypertension; The Barostim Hypertension Pivotal Trial (clinicaltrials.gov: NCT01679132) is currently in progress and aims to enroll 310 patients with resistant hypertension, randomised to receive optimal medical management alone or in combination with BAT (Ng, Saxena et al. 2016).

2.3.8.2 Carotid body modulation

Increased sensitivity of the peripheral chemoreceptors, located in the carotid bodies has been shown to increase BP (see Section 2.1.2.3.4), and carotid sinus denervation which disrupts the afferent signals from the carotid bodies, has been demonstrated to reduce SNA and BP in rats (McBryde, Abdala et al. 2013). A safety and feasibility study of

surgical, unilateral carotid body (CB) excision in humans, has now been conducted jointly by the CardioNomics research group in Bristol and Prof. Narkiewicz's group in Gdansk, Poland (Narkiewicz, Ratcliffe et al. 2016). In this study 8/15 patients responded with a reduction in ambulatory BP of ≥10 mmHg at 3 months post resection. BP rose back towards baseline by twelve months after surgery (Narkiewicz, Ratcliffe et al. 2016), but has fallen again at 24 months amongst those patients who responded to the procedure (unpublished data). In those that responded to CB excision, there was also a reduction in MSNA and an improvement in baroreflex sensitivity. Interestingly, before surgery, responders had a higher hypoxic ventilatory response and faster ventilatory frequency than non-responders, in keeping with a higher peripheral chemoreflex sensitivity and drive, respectively.

Work is now underway to develop a less invasive strategy for carotid body modulation. A trial is in progress to assess the feasibility of unilateral endovascular CB ablation in patients with resistant hypertension (clinicaltrials.gov: NCT02099851) (Ng, Saxena et al. 2016). A separate trial looks at alternative methods for achieving transient deafferentation of the CB by using local ultrasound-guided infiltration of lidocaine or local electrical stimulation to try to reduce BP (clinicaltrials.gov: NCT02519868) (Ng, Saxena et al. 2016). The carotid body may also be amenable to pharmacological modulation; pre-clinical data have demonstrated that antagonism of P2X3 purinergic receptors (which are present in the human CB) reduces BP and SNA, and normalises carotid body hyperreflexia in hypertensive rats (Pijacka, Moraes et al. 2016).

2.3.8.3 Central iliac arteriovenous anastomosis

Central iliac arteriovenous anastomosis employs a vascular, haemodynamic, as opposed to a sympathoinhibitory, approach to regulate BP. In patients with hypertension, the ROX Coupler device (ROX Medical, San Clemente, CA, USA) creates a 4-mm anastomosis between the iliac artery and vein, diverting a calibrated amount of arterial blood into the venous system (≈800 mL/min). The anastomosis reduces vascular resistance and the effective arterial circulating volume, and increases arterial compliance, resulting in an immediate and substantial reduction in both systolic and diastolic BP (see comprehensive review of the potential mechanisms of the antihypertensive effect of the ROX Coupler device (Burchell, Lobo et al. 2014)). In the ROX CONTROL HTN study, 42 patients were treated with the arteriovenous coupler therapy versus 35 control subjects: office SBP was reduced by 26·9 mmHg in the arteriovenous coupler group compared with a 3.7 mmHg reduction in the controls ($p<0·0001$), however, implantation of the ROX Coupler was associated with late ipsilateral venous stenosis in 29% of patients, although this was treatable with venoplasty or stenting (Lobo, Sobotka et al. 2015). 12-month follow-up data are now available for the ROX CONTROL HTN cohort, which report a 12.6 ± 17.4/15.3 ± 9.7 mmHg reduction in mean daytime ambulatory BP ($p<0.0001$ for both), however, 14/42 (33%) of patients developed iliac vein stenoses, although once again the authors reassure that these were successfully treated with venous stenting (Lobo, Ott et al. 2017). Further research is required to evaluate the long-term safety and efficacy of this technique.

2.3.8.4 Deep brain stimulation

The antihypertensive effect of deep brain stimulation (DBS) was initially reported as a serendipitous finding in cases of patients with concomitant hypertension, undergoing DBS for the treatment of chronic neuropathic pain (Green, Wang et al. 2007, Pereira, Wang et al. 2010, Patel, Javed et al. 2011). In animal models, stimulation of the ventrolateral periaqueductal grey elicits a depressor response and bradycardia via inhibition of sympathetic premotor neurons in the rostral ventrolateral medulla, and by improving baroreflex sensitivity through projections to the nucleus raphe magnus (O'Callaghan, McBryde et al. 2014). Recently, DBS has been reported to reduce BP and MSNA in a patient with severe hypertension resistant to pharmacotherapy, RDN and BAT (O'Callaghan, Hart et al. 2017). A small pilot study is underway in Bristol to further assess the safety and efficacy of DBS in patients with refractory hypertension.

2.3.8.5 Median and vagal nerve stimulation

Devices are also under development for the treatment of hypertension through stimulation of the median and vagal nerves. The rationale for median nerve stimulation is based on data from studies of electroacupuncture (Li, Tjen et al. 2015, Ng, Saxena et al. 2016). Valencia Technologies (Valencia, CA, USA) are developing an eCoin device for median nerve stimulation, with their unpublished interim results suggesting that following implantation, those with the device switched on (n=11) had an 11 mmHg reduction in BP vs the sham control group (device implanted but switched off, n= 10) who had a 4 mmHg increase in BP(2017).

Devices targeting autonomic modulation in hypertension have primarily aimed to achieve sympathoinhibition, but an alternative approach would be to consider parasympathetic excitation. Vagal nerve stimulation (VNS) devices have been developed focussing on the treatment of CHF. To date, randomised clinical trials using these devices in CHF have produced disappointing results, with high levels of adverse events, and a failure to demonstrate an improvement in cardiac remodelling or functional capacity (NECTAR-HF (Zannad, De Ferrari et al. 2015)), or the rate of death or heart failure related events (INOVATE-HF (Gold, Van Veldhuisen et al. 2016)), although NYHA classification did improve in the ANTHEM-HF (Premchand, Sharma et al. 2014) and INOVATE-HF trials (Smith, Rossignol et al. 2016). To date, there are limited data on the use of VNS in hypertension, with one case report describing an increase in BP (Sokolovic and Mehmedagic 2016), despite more positive pre-clinical findings (Ng, Saxena et al. 2016).

2.3.8.6 Carotid sinus remodelling

The MobiusHD (Vascular Dynamics, Mountain View, CA, USA) implant aims to reduce blood pressure by remodelling the carotid sinus. This endovascular, self-expanding rectangular cuboid implant is proposed to improve baroreflex sensitivity by increasing carotid sinus arterial wall strain (Spiering, Van Der Heyden et al. 2015). In the CALM-FIM (Controlling And Lowering Blood Pressure With The MobiusHD – First In Man) study, one month after implantation, patients treated with the MobiusHD device had a -21/-9 mmHg fall in office BP (n=15), sustained out to 12 months (-32/-19 mmHg, n=4) (Spiering, Van Der Heyden et al. 2015). CALM-DIEM, a single-arm, safety, efficacy study,

enrolling 200 patients (clinicaltrials.gov: NCT02827032), and CALM-START, a multicenter, prospective, randomized, blinded, sham-controlled study, enrolling 110 patients (clinicaltrials.gov: NCT02804087), are currently ongoing and recruiting subjects in Europe.

2.3.9 Conclusions

In less than 10 years the field of interventional therapy in hypertension has exploded from first-in-man studies of RDN, into a competitive market encompassing a range of catheter technologies and advancing into broader sympathoexcitatory indications. Other interventional strategies have built on the renewed interest in the autonomic mechanisms underlying hypertension, with most techniques aimed at harnessing the antihypertensive effect of sympathoinhibition. This story is however, a cautionary tale, with confusion over the interpretation of Symplicity HTN-3 as to whether RDN is ineffective at lowering BP, or whether failings in the study design affected its outcome. It is now time to go back and consider the physiological basis for RDN in more detail, and to confirm the efficacy of the procedure. Large scale outcome data are showing positive preliminary results from ongoing commercial studies, whilst this study aims to understand the procedural efficacy and autonomic effects on a mechanistic level, with focus on devising a test to ensure on table procedural efficacy, and if possible, understand whether RDN preferentially affects afferent or efferent renal nerves.

3 Study Aims, Hypotheses and Outcome Measures

Currently, when managing patients with resistant hypertension, it is difficult to provide evidence as to whether they are likely to benefit from renal denervation. In this pilot study, we aim to better understand RDN from the individual patient perspective, and to develop a test that will confirm denervation of renal nerves at the time of the procedure. When interpreting individual outcomes following RDN, it is impossible to know for certain whether any reduction in blood pressure (BP) after treatment is due to disruption of the renal nerves, unless renal nerve integrity can be assessed directly.

3.1 Aims of the study

3.1.1 Primary aim

To generate data that allow an accurate prediction as to whether a patient with resistant hypertension will respond to renal denervation therapy. We will achieve this by assessing a pattern of baseline markers of overactive sympathetic activity, heart rate variability, sympathovascular transduction[3], systemic inflammation, cerebral blood flow, and altered sensitivity of peripheral chemoreceptor and baroreceptor reflexes and correlate them with procedural success.

3.1.2 Secondary aim

To develop methods for quantifying the procedural success of renal denervation treatment that can be applied at the time of the procedure.

1. We will assess the function of
    a. the efferent sympathetic pathway by measuring reflex evoked changes in renal blood flow in response to a sympathetic stressor (hand grip exercise) using an intra-arterial Doppler flow wire
    b. stimulation of renal afferents with intra-renal artery adenosine infusion and measurement of reflex increases in BP.

3.1.3 Additional aims

1. To confirm the safety of renal denervation in the treatment of hypertension.
2. To assess the efficacy of RDN in reducing blood pressure and in reducing hypertensive target organ damage. This will be done by measuring the effect of RDN on office blood pressure, ambulatory blood pressure, renal function (e.g. estimated glomerular filtration rate, urinary albumin:creatinine ratio), aortic distensibility and indices of hypertensive cardiac remodeling, including left ventricular hypertrophy using magnetic resonance imaging (MRI).

[3] Sympathovascular transduction (also sympathetic-vascular coupling) describes the conversion of sympathetic nerve activity (peroneal muscle sympathetic nerve activity in this instance) into vascular tone, quantifying the efficacy of neuro-vascular transmission.

3. To better understand the mechanisms underlying the antihypertensive effect of renal denervation by assessing changes in sympathetic nerve activity, cardiac and sympathetic vascular baroreflex sensitivity, peripheral chemosensitivity, and systemic inflammation following renal nerve ablation.
4. To establish whether ablation of the afferent renal nerves, efferent renal nerves or both groups of nerves underpins the antihypertensive effect of renal denervation.

3.2 Experimental Hypotheses

3.2.1 Hypothesis for primary aim

- Patients with high levels of baseline sympathetic nerve activity, raised markers of systemic inflammation, abnormal cerebral blood flow, and altered peripheral chemoreceptor and baroreceptor reflex sensitivity will respond to renal denervation with a fall in BP in accordance with the primary outcome measure.

3.2.2 Hypotheses for secondary aim

- Measures which assess the function of the renal efferent sympathetic pathway by measuring reflex evoked changes in renal blood flow and the renal afferent pathway by looking at reflex changes in blood pressure following activation of the renal chemoreflex by intra-renal artery infusion of adenosine, will confirm the procedural success of RDN.
- The improvement in BP following RDN will correlate with the reduction in reflex responses to renal afferent and efferent nerve stimulation.

3.2.3 Hypotheses for additional aims

1. Renal denervation will exhibit an acceptable safety profile, in keeping with existing adverse event rates from previous published trial and registry data.
2. Renal denervation will reduce office and ambulatory blood pressure. In patients with a reduction in BP and/or sympathetic nerve activity there will be prevention of, or an improvement in, target organ damage.
3. Blood pressure reduction following renal denervation will be associated with a reduction in sympathetic nerve activity, systemic inflammation and peripheral chemoreceptor hypersensitivity, and an increase in baroreflex gain.
4. Blood pressure reduction following renal denervation will be dependent on disruption of both the afferent and efferent renal nerves.

Testing these hypotheses will enable confident selection of patients most likely to respond to RDN and interpretation of the outcome measures listed below.

3.3 Outcome Measures

3.3.1 Primary outcomes

- The change in office systolic BP and mean daytime ambulatory systolic BP at 6 months after the procedure will be measured. We define a responder as exhibiting a fall in office systolic BP of ≥10mmHg at 6 months post RDN. In Symplicity HTN-2, the primary outcome measure was the reduction in office systolic BP at 6 months post-RDN, with a response to RDN defined as a reduction in office systolic BP of ≥10 mmHg (Esler, Krum et al. 2010). The change in office systolic BP will be correlated against baseline:
  - muscle sympathetic nerve activity
  - heart rate variability
  - markers of inflammation
  - sympathovascular transduction
  - peripheral chemoreflex sensitivity and baroreflex gain
  - cerebral blood flow

3.3.2 Secondary outcomes

- For efferent procedural success, we will measure the change in renal vascular resistance in response to handgrip stress, before and after renal denervation, and see if the difference between these measures predicts change in BP at 1-month post-RDN.
- For afferent procedural success, we will measure the change in systemic systolic BP in response to adenosine infusion into the renal arteries, before and after renal denervation, and assess whether the difference between these measures predicts change in BP at 1-month post-RDN.

3.3.3 Additional outcomes

1. Measures of the safety of the procedure will be assessed by any complications or adverse clinical events during or after the renal denervation procedure. We will also closely monitor renal function and will assess for the development of renal artery stenosis at 6 months using magnetic resonance angiography.
2. Changes in office and ambulatory BP and measures of muscle sympathetic nerve activity, heart rate variability, sympathovascular transduction, baroreflex gain, peripheral chemoreflex sensitivity, cerebral blood flow, and markers of inflammation will be assessed at 1, 3, 6 and 12 months after the procedure (cerebral blood flow measured at baseline and 6 months only).
3. Renal function, microalbuminuria, aortic distensibility and left ventricular mass will be assessed prior to RDN and at 6 months to identify any regression of hypertensive end organ damage at 6 months.
4. Antihypertensive medications will not be altered during the study unless BP falls below 120/90mmHg or a patient develops significant symptomatic hypotension. Likewise, we will aim to avoid any increases in antihypertensive medications in which BP remains elevated, unless the participant experiences worsening symptomatic hypertension. If changes are essential, we will document any

changes in the number or dosages of antihypertensive medication in patients following RDN.

Outcome measures are summarised in Box 3.1. Physiological measures based on autonomic and pathologic markers were assessed at all study visits (0, 1, 3, 6, and 12 months), however, magnetic resonance imaging was only performed at baseline and 6 months post-RDN for logistical reasons.

Summary of outcome variables

Procedural safety

- Adverse event reporting
- Estimated glomerular filtration rate (eGFR)
- Renal magnetic resonance angiography

Procedural efficacy

- Number of ablations administered
- Reflex change in renal vascular resistance in response to handgrip stress
- Reflex change in systemic blood pressure in response to renal intra-arterial adenosine infusion

Response to renal denervation

- Office blood pressure
- Ambulatory blood pressure
- Antihypertensive medication: whole dose equivalents
- eGFR
- Urinary albumin:creatinine ratio
- Aortic distensibility
- Left ventricular hypertrophy
- Left ventricular strain
- Left ventricular interstitial fibrosis using T1 mapping (in selected patients)

Predictors of response to renal denervation (and mechanistic markers)

- Muscle sympathetic nerve activity
- Heart rate variability
- Sympathovascular coupling
- Cardiac and sympathetic vascular baroreflex sensitivity
  - including response to Modified Oxford testing in a subset of patients
- Hypoxic ventilatory response (peripheral chemoreflex sensitivity)
- Cerebral blood flow
- Inflammatory markers
  - Interleukin(IL)- 6, IL-8, IL-10, IL-17, myeloperoxidase (MPO), tumour necrosis factor (TNF)-α, C-reactive protein (CRP)

Box 3.1. Summary of outcome variables for the renal denervation for resistant hypertension pilot study.

4 Overall study design and general methods

This study investigated the changes in a range of physiological parameters in patients with resistant hypertension, before and after treatment with renal denervation. The study aimed to:

- improve patient selection for RDN
- devise a measure of procedural success which can be used at the time of the procedure to guide adequate renal nerve ablation
- better understand the mechanisms underlying the antihypertensive effects of RDN.

4.1 Subjects and recruitment

This study aimed to recruit 30 patients with resistant hypertension, meeting the criteria below. As an observational, pilot study assessing the feasibility of autonomic screening and on-table renal nerve testing in the context of renal denervation, a power calculation was not undertaken. Recruitment took place between March 2012 and December 2016, with all patients recruited through the tertiary, specialist Hypertension Clinic at the Bristol Heart Institute. Formal written consent was obtained from all patients screened and enrolled in the study. The study was approved by the South West – Frenchay Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki.

4.1.1 Target population

The target population for the study was patients with resistant hypertension on medical management. These patients were defined as having an office systolic blood pressure (oSBP) of >140 mmHg, were prescribed at least three anti-hypertensive medications at maximum tolerated dose and had no evidence of causes for secondary hypertension following thorough clinical assessment.

4.1.2 Inclusion criteria

The study inclusion criteria were resistant hypertension (as defined above) and a participant age range of 30-75 years.

4.1.3 Exclusion criteria

The study exclusion criteria were, body mass index (BMI) >35 kg/m^2, pregnancy or anticipation of pregnancy, palliative care/chemotherapy, anticipated life expectancy less than 12 months due to other disease, renal transplant patients, renal function impairment (eGFR<45ml/min/1.73m^2), heart failure with reduced ejection fraction, severe cardiac valvular disease, acute coronary syndrome or unstable angina, untreated obstructive sleep apnoea, intravenous drug use, and an alcohol intake >28 units/week.

Patients with variant anatomy of the renal artery (e.g. early renal artery bifurcation, small renal arteries <4mm diameter, short renal arteries <20 mm, aberrant renal artery morphology (Esler, Krum et al. 2010)), which makes the patient unsuitable for renal artery denervation, were also excluded. Patients with a BMI of >35 kg/m^2 were excluded since obesity increases sympathetic nerve activity, particularly in males (Brooks, Shi et al. 2015), and therefore aimed to reduce confounding due to potential weight loss.

There were also specific magnetic resonance imaging (MRI) related exclusion criteria, including the presence of a pacemaker, implantable cardiac defibrillator, cerebral metallic clips or other implanted metal devices/structures. Additionally, participants unable to tolerate the scanner or with a history of panic attacks/claustrophobia, and participants with a learning disability, or significant hearing or visual impairment (participant would need to be able to communicate from within the MRI scanner) were excluded from MRI. If patients had a contraindication to MRI, then renal anatomy was determined using CT angiography of the renal arteries.

4.2 Study Design

The overall study design is summarised in Figure 4-1. This was a single centre, open-label, observational, pilot study, therefore patients were not subject to randomisation. Briefly, the patients attended a screening visit with an additional MRI session, and, if enrolled into the study, underwent renal denervation with on-table renal nerve testing. A significant ethical amendment was approved in December 2012, permitting the 'on-table' measures of procedural success. These measures form a sub-study in patients recruited from 2013 onwards and are reported in Section 5.5.

Participants attended for study visits at 0, 1, 3, 6, and 12 months, involving detailed autonomic profiling. The format of these study visits is summarised in Figure 4-2. The protocol for the blood and urine samples taken at each study visit is summarised in Table 4-1. It was not possible to obtain fasting samples for logistical reasons; several participants lived a long way from the Bristol Heart Institute, requiring study sessions in the afternoon, and therefore could not be expected to fast for a prolonged period. Participants were advised to have an early light breakfast or lunch before morning or afternoon study sessions, respectively. Study sessions were held at the same time of day for any given patient. Patients were advised to abstain from alcohol and caffeine on the day of a study visit and to take their prescribed medication at the usual times.

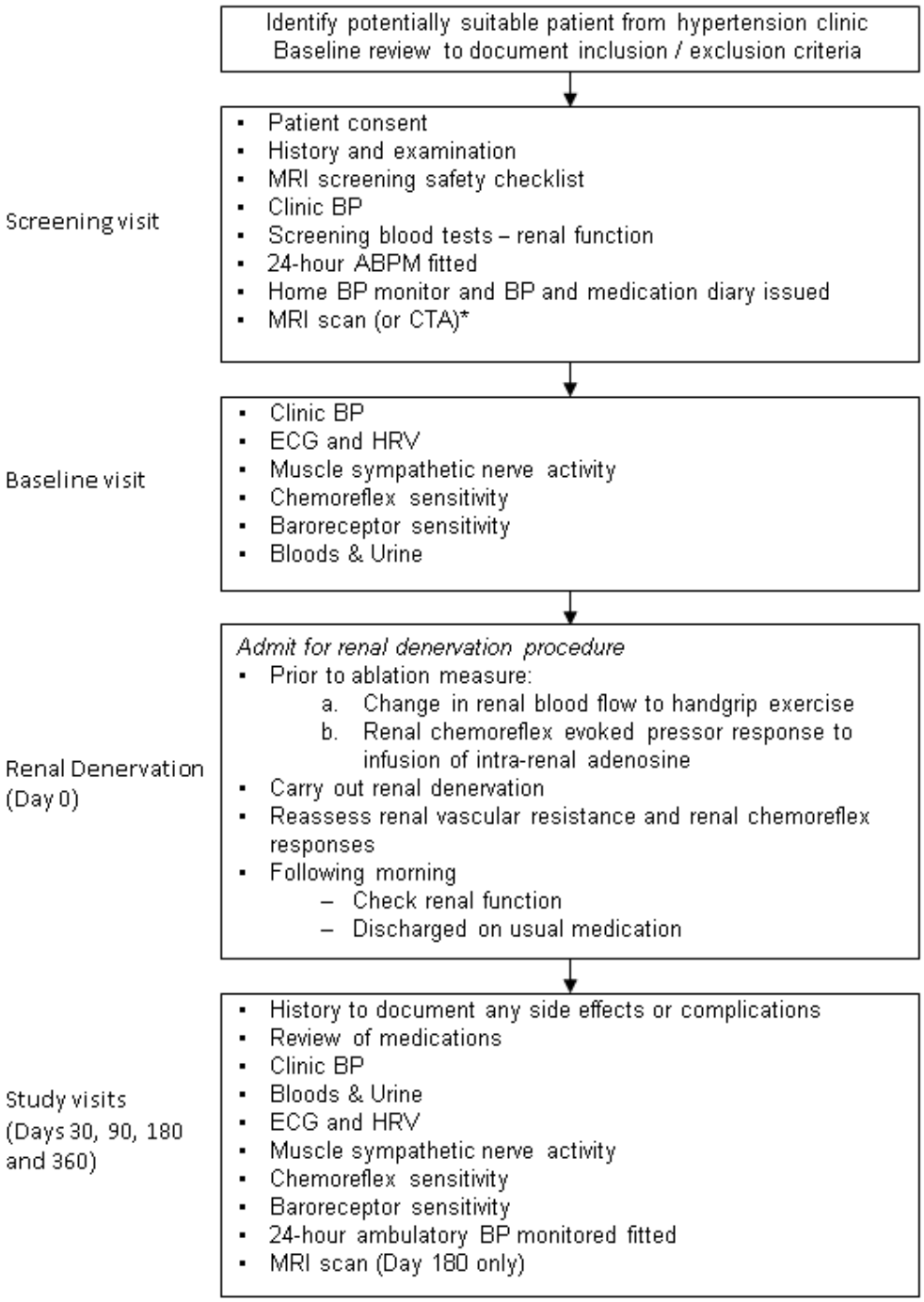


Figure 4-1 Summary of the overall study design for the renal denervation for resistant hypertension pilot study.

* Or renal computerised tomography angiogram (CTA) if magnetic resonance imaging (MRI) contraindicated. BP, blood pressure; ABPM, ambulatory BP monitoring; ECG, electrocardiography; HRV, Heart Rate Variability.

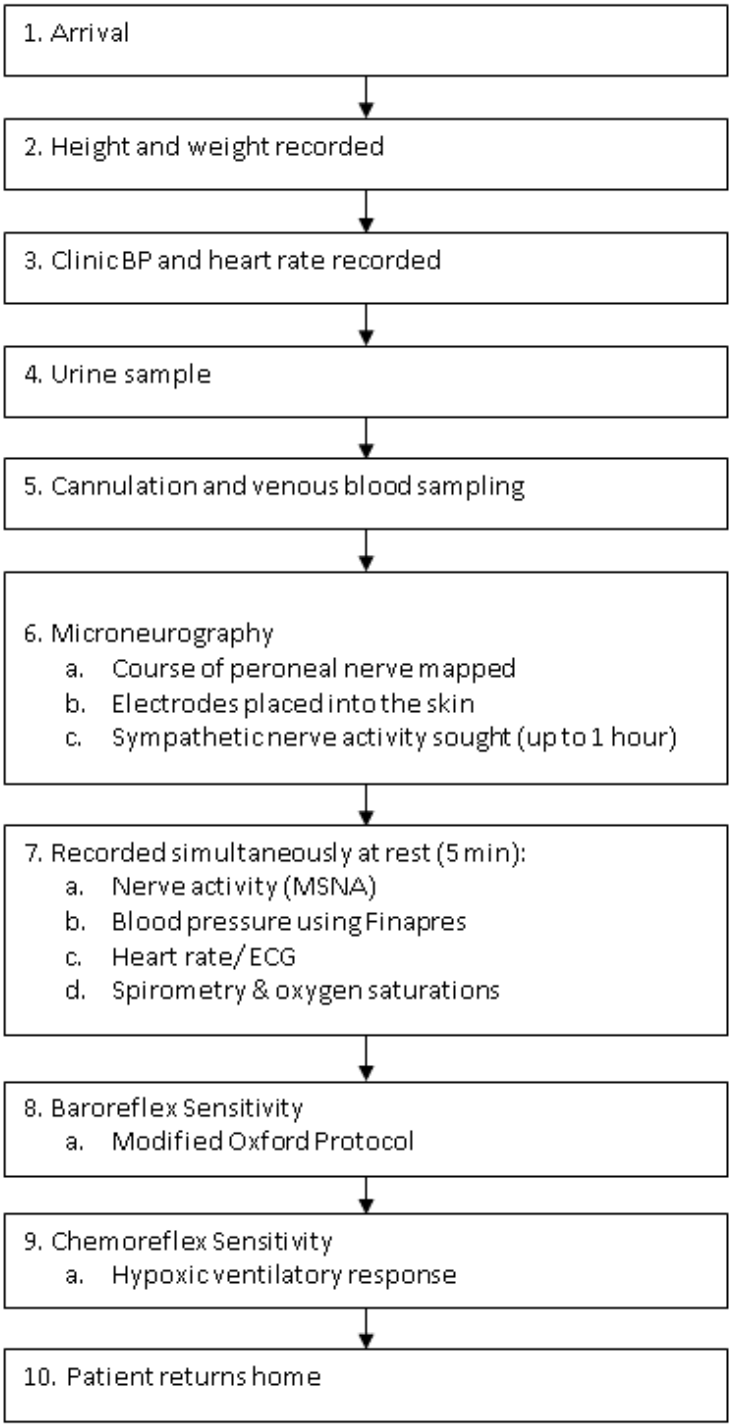


Figure 4-2. Study day schedule.
Detailed methods for these investigations can be found in the general and specific methods sections. The full study visit lasts approximately 3 hours. BP, blood pressure; MSNA, muscle sympathetic nerve activity; ECG, electrocardiogram.

Test	Screening	Baseline	1 month	3 months	6 months	12 months
FBC		X	X	X	X	X
U&E	X	X	X	X	X	X
Lipid profile		X			X	X
HbA1c		X			X	X
CRP		X	X	X	X	X
Fibrinogen		X	X	X	X	X
MPO		X	X	X	X	X
TNFα		X	X	X	X	X
IL6		X	X	X	X	X
IL8		X	X	X	X	X
IL10		X	X	X	X	X
IL17		X	X	X	X	X
Imm cells		X	X	X	X	X
ACR*		X			X	X

Table 4-1. Blood and urine sample schedule.
*Urine sample. FBC, full blood count; U&E, urea and electrolytes; HbA1c, haemoglobin A1c; CRP, C-reactive protein; MPO, myeloperoxidase; TNFα, tumour necrosis factor alpha; IL, interleukin; imm cells, immune cell profiling; ACR, albumin:creatinine ratio.

4.3 General methods

The methods for basic investigations referred to repeatedly in the body of this thesis are described below. Detailed methods of more specialist tests are described in Chapter 5, Specific Methods and Results.

4.3.1 Baseline demographic data

Baseline demographics and clinical characteristics were recorded. A detailed hypertensive and past medical history was obtained. Prescribed medications were documented and whole dose equivalents (WDE; percentage of maximum licensed dose of prescribed medication) were calculated (Antoniou, Saxena et al. 2016). Patient height and weight were measured with calculation of body mass index (BMI (kg/m^2) = height /$weight^2$).

4.3.2 Blood pressure monitoring

4.3.2.1 Clinic/office BP readings

A validated oscillometric BP monitor (Omron, Kyoto, Japan), with an appropriately sized cuff, was used, and readings were recorded with the subject seated at quiet rest for five minutes. BP readings checked in both arms at first attendance, and three BP readings, two minutes apart, from the arm with higher level were recorded; the final two readings were averaged to give the mean systolic and diastolic office BP. The resting heart rate was also recorded.

4.3.2.2 Ambulatory BP monitoring

A validated oscillometric ambulatory BP monitor was used (Spacelabs Healthcare, OSI Systems, Hawthorne, CA, USA) with an appropriately sized cuff (NICE 2015). The device was left on for a 24-hour period to encompass a 'routine' day. BP readings were acquired every 30 minutes during the day and every 60 minutes overnight, and the subject was advised to pause and support the cuffed arm during readings if possible. For the purposes of this study daytime was defined as 0600-2159 hours and night-time was defined as 2200-0559 hours.

4.3.2.3 Home BP monitoring and medication diary

The use of a home BP diary, including self-reporting of medications taken, also aimed to act as an indirect measure of compliance (similar approach to Symplicity HTN-2 study design, (Esler, Krum et al. 2010)). A validated oscillometric BP monitor (Omron, Kyoto, Japan), with an appropriately sized cuff, was given to the patient, along with appropriate instruction, for use at home.

After sitting in quiet room for 5 minutes, measurements were taken from the arm with higher clinic reading, four times a day for eight days. At each measurement session, subjects were advised to take three readings, with three minutes rest between each reading. Results from the first day (practice readings) were discarded and an average was taken of the second and third BP readings for all timepoints over the remaining seven days, and then used to calculate an overall mean home BP result. Participants were also asked to document medications taken during the day, and data were collected using a standardised home BP diary.

4.3.3 Blood and urine samples

A venous cannula was sited in the right antecubital fossa or forearm, and around 40 ml of blood was obtained via the cannula for analysis. This was performed at room temperature, with the patient at seated rest. The patients were not in a fasted state. A mid-stream urine sample was also collected. The analyses performed during the study are summarised in Table 4-1. Routine tests were performed via the Pathology Department at the Bristol Heart Institute. Serum samples were frozen for subsequent analysis of inflammatory markers by Dr Tanya Smith at the University of Bristol. All blood and urine analysis were undertaken by researchers blinded to the study outcome to minimise bias.

4.3.4 Magnetic resonance imaging

Baseline magnetic resonance images were acquired to define the anatomy of the renal arteries. Patients with variant renal artery anatomy (e.g. early renal artery bifurcation, small renal arteries <4 mm diameter, short renal arteries, aberrant renal artery morphology) or renal artery stenosis were excluded from this study in keeping with Joint Society UK guidelines (Lobo, de Belder et al. 2015). MRI was also used to assess left

ventricular mass (LVM) and fibrosis. A more detailed evaluation was performed in patients enrolled in this Renal Denervation for Resistant Hypertension pilot study, which were beyond the scope of the routine clinical Hypertension MRI protocol, including quantification of left ventricular (LV) strain and cerebral blood flow (CBF) (see Sections 5.2.2.6 and 5.4.4.2 respectively for full methodology). Patients with an eGFR <45ml/min/1.73m^2 were excluded from the study as there is a relative contraindication to using gadolinium in this group of patients due to an increased risk of nephrogenic systemic fibrosis (Prince, Zhang et al. 2008).

4.3.4.1 MRI protocol

Our clinical Hypertension MRI protocol has previously been published (Burchell, Rodrigues et al. 2017). Images were acquired from the level of the Circle of Willis to the level of the femoral heads.

Cardiac MRI (CMR) was performed at 1.5 Tesla (Avanto, Siemens, Erlangen, Germany). Steady-state free precession (SSFP) short-axis whole LV cines (8 mm slice thickness, no slice gap, temporal resolution 38.1 ms, echo time 1.07 ms, representative field-of-view (FOV) in-plane pixel size 1.5 × 0.8 mm) were used for the estimation of LV mass and volumes. Myocardial replacement fibrosis was assessed by late gadolinium enhancement (LGE) (Mahrholdt, Wagner et al. 2005). An inversion-recovery fast gradient-echo sequence was performed 10–15 min after intravenous administration of 0.1 mmol/kg gadobutrol (Gadovist, Bayer Pharma AG, Germany), in two phase-encoding directions where there was potential artefact. Individually tailored inversion times were used in each patient to null normal reference myocardium.

Renovascular assessment consisted of Time-resolved angiography with Interleaved Stochastic Trajectories (TWIST) contrast enhanced magnetic resonance angiography (MRA), which creates multi-phase, multi-planar images of the thoracic and abdominal vasculature; angiography was analysed using multiplanar reformatting post-processing software (cmr42; Circle Cardiovascular Imaging Inc., Calgary, AB, Canada). Axial T1-weighted images through the abdomen and pelvis with 5mm slice thickness were also performed.

4.3.4.2 CMR analysis

Blinded MRI analysis was performed by Dr Nathan Manghat and Dr Jonathan Rodrigues at the Bristol Heart Institute Cardiac Magnetic Imaging facility. The assessment of left ventricular volumes and LVM were performed as described previously (Maceira, Prasad et al. 2006). Briefly, endocardial contours were defined at end-diastole and end-systole on the LV short-axis stack using blood pool/endocardial border threshold detection software (cmr42; Circle Cardiovascular Imaging Inc., Calgary, AB, Canada), which has been previously validated (Childs, Ma et al. 2011). Epicardial contours were defined manually at end diastole. The LVM was estimated by multiplying the total myocardial volume, including papillary muscles and LV trabeculations (equivalent to LV dry weight), by 1.05 g/ml, which is the specific gravity of myocardium, as described previously (Maceira, Prasad et al. 2006). The LVM was indexed to body surface area, calculated using the Mosteller formula. Ejection fraction was calculated from the end-diastolic and

end-systolic endocardial volumes, and long axis function was assessed using mitral and tricuspid annular plane systolic excursion.

LVH was defined as indexed LVM >95th percentile of established CMR reference ranges indexed to body surface area (men: <35 years, >87 g/m^2; ≥35 years, >78 g/m^2 and women: <35 years, >71 g/m^2; ≥35 years, >70g/m^2) (Hudsmith†, Petersen† et al. 2005). Left ventricular mass is indexed to body surface area, with gender specific cut-offs, to account for three different sources of physiologic variation: lean body mass, obesity, and gender (Foppa, Duncan et al. 2005). LV remodelling was defined as a ventricle with normal indexed LVM but elevated LV mass/volume ratio (M/V) (Buchner, Debl et al. 2009). An increased M/V was defined as >95th gender-specific percentile (men: >1.12 g/ml and women:>1.14 g/ml) from healthy volunteers, as described previously (Buchner, Debl et al. 2009). The presence of LGE was quantified by visual analysis.

4.3.5 Physiological assessment

At the baseline and follow-up study visits a range of physiological parameters were measured at rest to help to establish an autonomic profile for each patient.

Baseline measurements were taken over 5 to 10 minutes, with the patients lying supine, at quite rest. Simultaneous readings were made of the parameters detailed below. The data were collected via a PowerLab (AD Instruments, Dunedin, New Zealand) and recorded continuously using a data acquisition program on a study laptop (LabChart, AD instruments, Dunedin, New Zealand). The resting variables included (see Figure 4-3); beat-to-beat blood pressure measured using a Finometer device (Finapres Medical Systems, Enschede, Netherlands), heart rate (and rhythm) from a 3-lead electrocardiogram (ECG) recording, non-invasive oxygen saturations (Radial-7, Masimo Corp., Irving, CA, USA), chest excursion using a respiratory belt, and measurement of muscle sympathetic nerve activity (MSNA). Heart rate variability, spontaneous cardiac and sympathetic baroreflex sensitivity, and sympathovascular transduction were all calculated from the above parameters.

Further to this, baroreflex sensitivity was tested in response to BP modulation via injections of sodium nitroprusside and phenylephrine (the Modified Oxford test), and peripheral chemoreflex testing included the use of spirometry, and the measurement of end tidal carbon dioxide (ETCO2) and the fraction of inspired oxygen (FiO2). More detailed methods for these dynamic assessments are found in Sections 5.4.1.2.2 and 5.4.3.2, respectively.

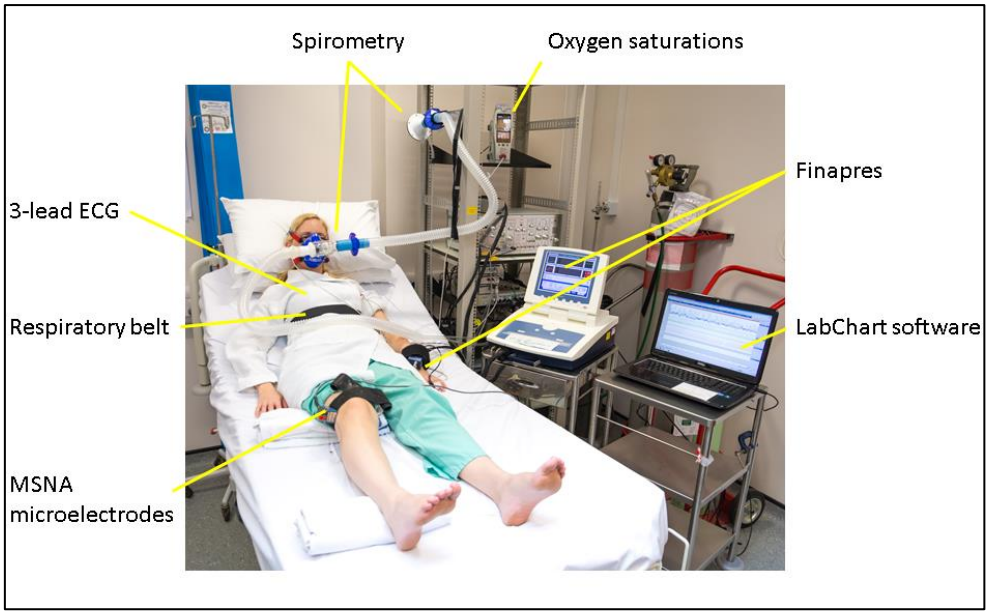


Figure 4-3. Illustration of equipment used for the autonomic assessment of patients during the renal denervation study.
Healthy volunteer shown.

4.3.6 Microneurography

4.3.6.1 Background

Sympathetic nerve activity can be measured in humans using a technique called microneurography. The technique of microneurography is a minimally invasive technique to record nerve activity in humans (Vallbo, Hagbarth et al. 2004). The first microneurography recording were made by Hagbarth and Vallbo in Uppsala, Sweden in 1965–1966 (Vallbo, Hagbarth et al. 2004). Work initially focussed on recording multiunit activity from the large muscle-spindle afferents (Vallbo, Hagbarth et al. 2004), however, investigators soon realised the wider potential of the technique, and sympathetic neural recordings were documented from nerves containing muscle afferent, muscle efferent, skin afferent, and skin efferent fibres (Hagbarth and Vallbo 1968, Vallbo and Hagbarth 1968, Wallin, Sundlof et al. 1981, Hart, Head et al. 2017).

Multiunit MSNA recordings are obtained from the integrated neurogram, quantifying the firing of multiple nerve fibres, which demonstrate burst synchronicity in relation to the cardiac cycle (example shown in Figure 4-4) (Hart, Head et al. 2017). It is also possible to make recordings from individual nerve fibres, or more correctly, recordings in which a single fibre predominates, identified from a characteristic waveform morphology (Macefield, Wallin et al. 1994, Hart, Head et al. 2017). It has been suggested that multiunit neurograms provide a measure of the sympathetic vasoconstrictor activity to all muscle beds, since multiunit MSNA recorded from different limbs within a subject demonstrate similar behaviour (Hart, Head et al. 2017).

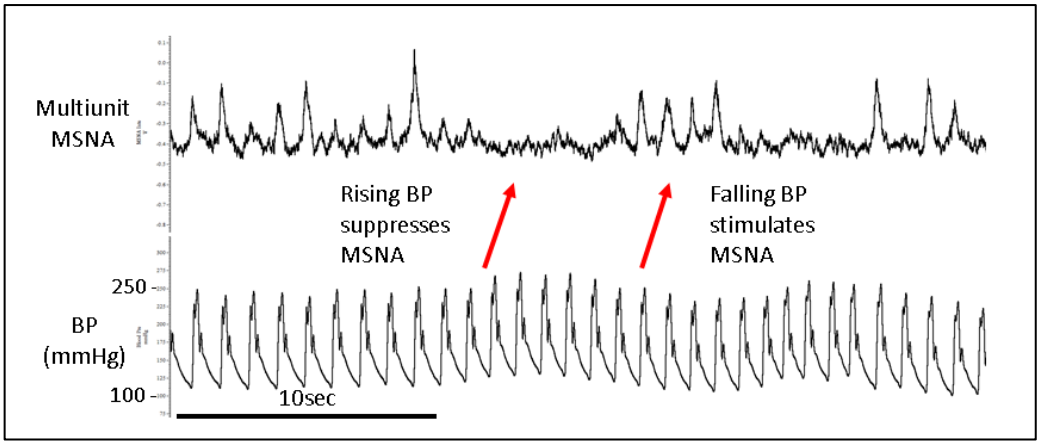


Figure 4-4. Integrated neurogram showing multiunit muscle sympathetic nerve activity recording (MSNA) and the relationship between MSNA and spontaneous fluctuations in blood pressure (BP).
Sample of an integrated neurogram recording from participant from this cohort at semi-supine, quiet rest. In addition to the baroreflex modulation shown in the figure, with MSNA suppressed by rising BP, MSNA bursting is pulse modulated, with bursts occurring when baroreceptors are offloaded in diastole. As can be seen from the figure, there is a latency between a change in diastolic BP and a change in MSNA due to the time for reflex transmission.

Single-unit recordings provide information about the temporal characteristics of the firing of that fibre, including, how often a fibre is active, whether the fibre fires multiple times in a burst and whether or not the fibre fires between bursts. These recordings also provide information about how the fibre reacts to internal (e.g. BP or respiration) and external stimuli. Due to the technical difficulties in obtain a stable single-unit recording for more than 3-5 minutes, good quality single-unit recordings are more challenging to obtain as a measure of resting MSNA (Hart, Head et al. 2017).

In this study we present data from multiunit recordings, and henceforth MSNA refers to multiunit MSNA.

MSNA had been shown to have excellent intraindividual reproducibility, however there is high interindividual variability, making it difficult to define a valid normal range (Fagius and Wallin 1993, Vallbo, Hagbarth et al. 2004). It is also important to note that MSNA tends to increase with age and is also affected by gender, oestrus cycle and the state of mind further complicating interpretation (Hart, Charkoudian et al. 2009). Despite these limitations, resting MSNA has been demonstrated to be elevated in hypertension (Yamada, Miyajima et al. 1989), and given the intraindividual reproducibility, a change in MSNA following RDN in an individual patient is likely to represent a true physiological effect.

4.3.6.2 Technique

Muscle sympathetic nerve activity (MSNA) is recorded from the peroneal nerve in the lower leg; this is the one of most superficial nerves in the human body which facilitates recordings as it is easy to locate. MSNA is conducted by post-ganglionic neurones with

small C-fibre (unmyelinated) axons at a velocity of about 1 m/s, and multiunit activity from these neurones are quantified as a measure of MSNA (Fagius and Wallin 1980).

The microneurography procedure utilises two tungsten micro-electrodes (FHC Inc, Bowdain, ME, USA), these have a very fine tip which is less than the width of a human hair (< 1 micron). The reference electrode (non-insulated) was inserted into the surface of the skin around 1-3 cm from the course of the peroneal nerve. The active electrode (insulated shaft, non-insulated tip) was inserted into the peroneal nerve to record nerve activity, locating a site in the nerve yielding discrete arterial pressure pulse-synchronised MSNA bursts (White, Shoemaker et al. 2015, Hart, Head et al. 2017). The location of the peroneal nerve was established using anatomical landmarks over the head of the fibula and a non-invasive, transcutaneous, stimulating electrode (see Figure 4-5). A muscle sympathetic fascicle was identified when taps on the tibialis anterior muscle belly or passive muscle stretch via flexion of the toes or inversion/eversion of the foot induced mechanoreceptive impulses. Bursts of neural activation associated with sensory stimuli were excluded by testing for response to light touch or a sudden loud noise/startle, which stimulate sensory nerves and skin SNA respectively (White, Shoemaker et al. 2015, Hart, Head et al. 2017). The recorded signal was amplified 80,000-fold, band pass filtered (700 to 5000 Hz), rectified and integrated (time constant 0.1 s) by a dedicated amplifier (Absolute Design and Manufacturing Services, Iowa, USA).

Prior to microneurography, participants were asked to abstain from alcohol and caffeine for 24hrs as this are known to impact MSNA (Randin, Vollenweider et al. 1995, Corti, Binggeli et al. 2002). Recordings were made at the same time of day in each participant with only a light meal prior to recordings (Cox, Kaye et al. 1995), and subjects were advised to take their antihypertensive medication at the usual time. Participants were asked to void their bladder immediately before this physiological testing since a full bladder can increase MSNA (Fagius and Karhuvaara 1989), and background noise was kept to a minimum during the recordings. Following instrumentation, 5-10 minutes (minimum 5 min) of resting baseline data were collected with the patients lying semi-supine at quite rest (but not asleep), with simultaneous, continuous measurement of: beat-to-beat BP (finger plethysmography), 3-lead ECG, MSNA, non-invasive oxygen saturations and chest excursion using a respiratory belt. Data were sampled at 1kHz (LabChart, AD Instruments, Dunedin, New Zealand) and stored on a personal computer for off-line analysis.

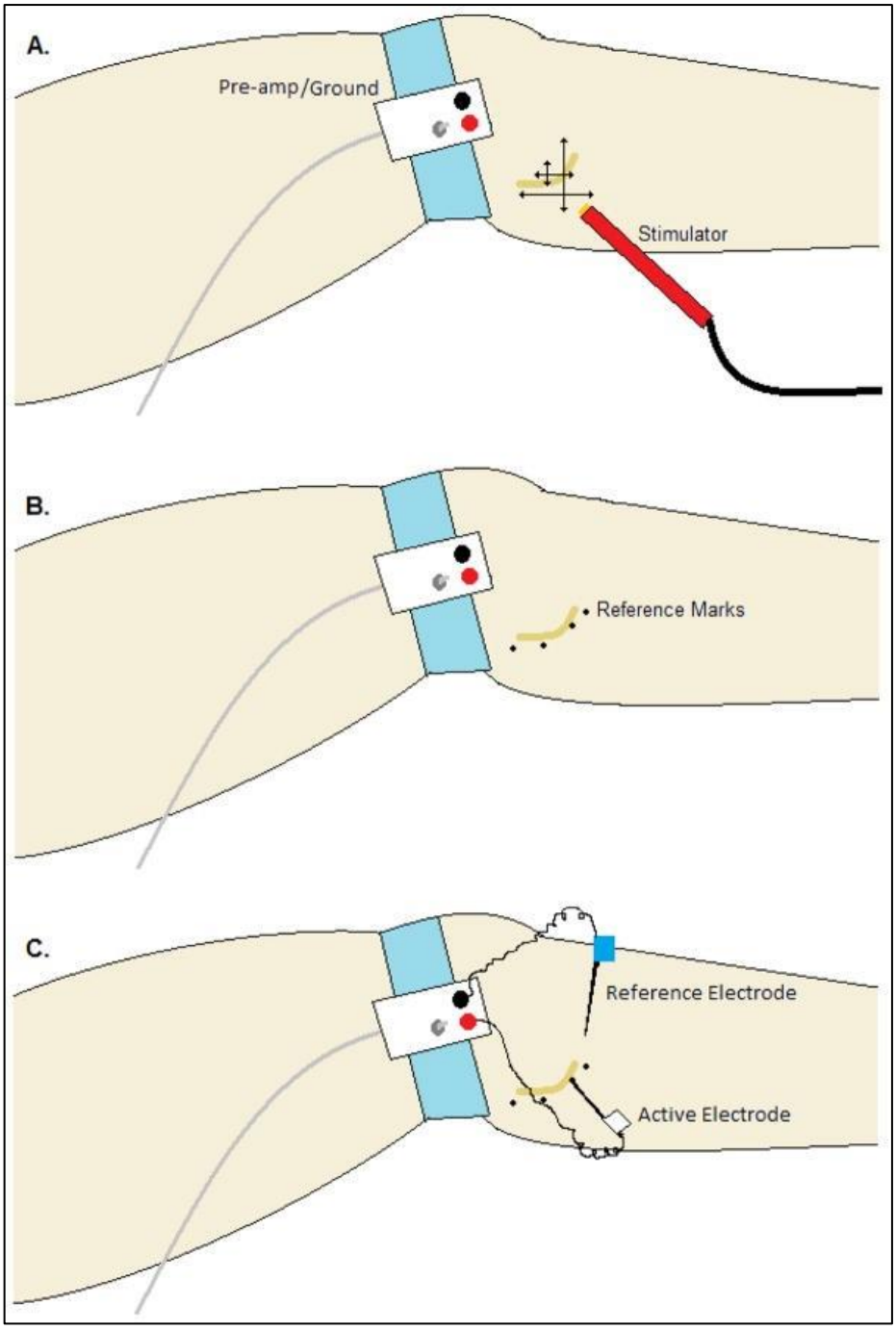


Figure 4-5. Method for identifying the course of the peroneal nerve and positioning the electrodes for the measurement of muscle sympathetic nerve activity.
The pre-amplifier and ground were attached to the skin on a flat surface at the lateral knee joint with the leg supported by a wedge and flexed at approximately 30^{o} to facilitate access to the peroneal nerve. The fibula head was identified using bony landmarks which can be palpated on the lateral aspect of the leg just below the knee. A) The systematic pattern of cutaneous electrical stimulation to locate and map the path of the nerve. B) The dots placed on the skin following the path of the nerve acted as a reference for insertion of the recording electrode. 3) The reference electrode (blue) was placed beneath the skin and into the tissue within 1-3 cm of the expected recording site. The active electrode (white) was inserted through the skin and manipulated until a satisfactory nerve signal was acquired. (White, Shoemaker et al. 2015)

4.3.6.3 Safety

Microneurography has been completed in 100s of studies worldwide (PubMed search: human microneurography, 827 citations, March 2018), both in normal healthy participants and in patients with neuronal disorders and cardio-respiratory diseases (Sundlof and Wallin 1978, Sundlof and Wallin 1978, Wallin 1978, Wallin and Eckberg 1982, Eckberg, Wallin et al. 1989, Charkoudian, Joyner et al. 2006, Hart, Charkoudian et al. 2009, Hart, Joyner et al. 2009).

Eckberg et al. have published a prospective study of symptoms occurring after microneurography (Eckberg, Wallin et al. 1989). The study followed 1000 patient recordings and found minor after-effects, such as deep transient aches in specific muscles (onset usually 2-3 days after microneurography with resolution within 3-7 days), in less than 10% of the studies. Eckberg et al. reported only one major adverse event which was a case of small fibre neuropathy. Based on their data, Eckberg et al. recommend that the time manipulating the active microelectrode to look for nerve activity should be limited to 1 hour to minimise adverse effects.

4.3.6.4 Data analysis

Sympathetic bursts (MSNA) in the integrated neurogram comprising multiple units were identified by a custom-written script (Dr E. Hart, University of Bristol, UK) using Spike 2 software (Cambridge Electronic Design Ltd, Cambridge, UK), requiring bursts that are ≥2 standard deviations above the noise, where bursts occur ~1.3 s (and not <0.9 s) after the previous R wave, based on average latencies observed in humans with a conduction velocity of ~1m/s (Fagius and Wallin 1980, Wallin, Burke et al. 1994). Burst identification was checked by visual inspection by a trained investigator (Dr A. Burchell, unblinded), with the burst morphology and burst latency (delay from preceding R wave, mean latency ±0.2 s) taken into consideration (Hart, Head et al. 2017). It is also important to note that there is only one burst of MSNA per cardiac cycle, and that skin bursts are usually broad with a more variable morphology when compared with MSNA. Burst latency varies between individuals relative to a mean latency of ~1.3 s, particularly affected by the size of the subject (reflecting the time taken for the impulse to travel to the level of the fibula head), and is associated with the size of the multi-unit burst, with larger bursts having faster conduction velocities and therefore a shorter latency (Hart, Head et al. 2017). The inverse correlation between burst amplitude and burst latency may be related to what is termed the “size principle” where recruitment of faster conducting, bulbospinal fibres may occur as the intensity of the sympathetic burst becomes greater (Wallin, Burke et al. 1994).

MSNA data were expressed as burst frequency (bursts per minute) and burst incidence (bursts per 100 heart beats). Burst area was also calculated, requiring manual marking of the start and end of each burst, with integration of the neurogram subtended by these markers (custom script by Dr L. Briant and Dr E. Hart). The absolute signal to noise of a burst depends on its proximity to the active electrode, therefore the maximal area (amplitude) of the biggest spontaneous burst in each dataset was identified, and the strength of all other bursts are expressed as a percentage of this value, giving a burst area in units of %/s (Briant, Burchell et al. 2016). Measures of total area per minute, and total area per 100 heart beats were calculated (Hart, Head et al. 2017). Caution must be

exercised when compared burst amplitude/area data between different recording, even if obtained from the same individual at different times, due to unquantifiable differences in the proximity of the nerve fibres from the recording electrode with separate instrumentations (Hart, Head et al. 2017).

A selection 15/56 (27%) of neurograms were independently analysed by a second operator (Dr E. Hart), who was blinded to the BP outcome, as a quality control measure. The mean coefficient of variation for interobserver variability was 7.0% (within subject coefficient of variation = standard deviation/mean*100). A selection of 5/56 (9%) baseline MSNA recordings were analysed twice by the author, with a >12-month interval been analyses. The mean intra-observer coefficient of variation was 3.8%. This degree of intra- and interobserver variability is within the generally accepted coefficient of variation of <10%.

4.3.7 Renal denervation protocol

Renal denervation was performed in a cardiac catheter laboratory, using standard aseptic techniques. The patients were treated under conscious sedation using intravenous midazolam, with intravenous fentanyl administered as analgesia, since active ablation energy delivery was associated with diffuse visceral pain; the dosages of medication used are summarised in Figure 5-43. There was a designated analgesia nurse throughout the procedure to monitor the patient's level of pain and administer further analgesia and sedation if required.

Participants were given intravenous heparin to achieve an activated clotting time (ACT) of more than 250 seconds; the ACT was rechecked during the procedure, and further heparin administered if required. Femoral arterial access was achieved using a 6 French vascular sheath, and the renal arteries were accessed using a 6 French guide catheter. The procedure was performed under fluoroscopic guidance, and prior to cannulation of the renal arteries, an angiogram was performed of the aorta at the level of the renal arteries, in order to confirm suitable renal artery anatomy for denervation, and to provide a route-map for the procedure. Physiological measurements to assess afferent and efferent renal nerve integrity were then performed as detailed in Section 5.5.2.

For the renal denervation procedure itself, the Symplicity catheter (Flex or Spyral, Medtronic, Santa Rosa, CA, USA) was advanced into the renal artery and connected to a radiofrequency generator. For the Symplicity Flex catheter (n=17), four-to-six discrete, low-power radio frequency ablations, lasting up to 2 minutes each, and of 8 watts or less, were applied along the length of both main renal arteries, at ≥0.5 cm intervals, in order to achieve ablation of all four circumferential quadrants of the artery. During ablation, the catheter system monitored tip temperature and impedance, altering radiofrequency energy delivery in response to a predetermined algorithm (Krum, Schlaich et al. 2009, Esler, Krum et al. 2010). The Symplicity Spyral catheter (n=2), consists of a 4-electrode array mounted on a 4F catheter that self-expands into a helical configuration with electrodes located at 5 mm intervals, at 90° from each other circumferentially. Using the Spyral catheter, radiofrequency energy treatment was then delivered simultaneously to all 4 renal artery quadrants for 90 seconds (Kandzari, Kario et al. 2016). This process was repeated 2-3 times in each renal artery, giving up to 12 ablations per vessel. When present, accessory renal arteries of ≥4 mm in diameter were

treated in a similar fashion to the main renal arteries. Branch renal artery ablation was not performed as a part of this study. The number of successful ablation points was quantified for each renal artery treated. All RDN procedures were performed by the same operator, Prof. Andreas Baumbach (consultant interventional cardiologist), who was experienced in the technique and had undergone formal training and proctoring by Medtronic.

We acknowledge that a switch from the Symplicity Flex to the Symplicity Spyral catheter during the course of the study is potentially a confounding factor when assessing the response to RDN. We had aimed to use the Flex catheter throughout the study, but due to the long period of recruitment, this catheter was no longer commercially available for the final two study participants, having been withdrawn by the manufacturer in favour of the second generation Spyral device.

4.3.8 Assessment of efferent and afferent renal nerve integrity

Renal nerve integrity was assessed at the time of RDN, aiming to establish a method for quantifying successful renal nerve ablation, in a sub-study of measures of procedural success. Briefly, efferent nerve integrity was assessed by the reflex change in renal vascular resistance in response to a handgrip stressor, and afferent renal nerve integrity was assessed as the reflex increase in systemic blood pressure in response to renal arterial adenosine infusion. These measures were made immediately before and after denervation in the first, and then second, renal artery treated, and are described in detail in Section 5.5.2.

4.4 Statistics

Baseline demographic data are presented as mean ± standard deviation and all subsequent outcome data are presented as mean ± standard error of the mean (SEM). Where data is mean ± standard deviation has been used it is clearly indicated, and data should be considered as mean ± SEM unless otherwise specified. Prior to statistical analysis, data were assessed for normality using a Shapiro-Wilk test. An α value of $p<0.05$ was taken to indicate statistical significance throughout the course of the study.

Primary outcome data, comparing baseline and 6-month results, were analysed using a paired Student's T-test (or Wilcoxon matched-pairs signed rank test for non-parametric data). Data were also assessed across multiple study timepoints (0, 1, 3, 6 and 12 months); when normally distributed, differences between these outcome measures were assessed using a repeated-measures ANOVA with a between group Bonferroni Multiple Comparison Test. Where data failed the test for normal distribution, they were assessed using a Friedman Test, with Dunn's Multiple Comparison Test (GraphPad Prism, GraphPad Software Inc. La Jolla, CA, USA). To enable analysis on the basis of repeated measures where there were gaps in the data for a particular follow-up visit, the missing data were replaced with the outcome from the previous visit (data carried forward) giving the assumption that there was no change in that variable during the intervening time period.

Univariate correlations were performed using Pearson's correlation coefficient or Spearman's rank correlation for normally distributed and non-normally distributed data, respectively, with additional linear regression.

4.5 Summary

These general methods apply across all the different sections of the study protocol. Detailed descriptions of the methods used for further physiological analyses can be found in Chapter 5, Specific methods, results and conclusions.

5 Specific methods, results and conclusions

5.1 Screening and recruitment

5.1.1 Introduction

The efficacy of renal denervation, and study design in this field, are subject to ongoing clinical debate (Esler and Guo 2017), with further stimulus from the publication of interim results from the SPYRAL HTN-ON MED and -OFF MED studies (Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018). In the UK, the current Joint Society Consensus advises that given the lack of definitive evidence for the procedure, RDN should only be performed as part of a formal clinical trial to ensure the appropriate, ethical treatment of patients (Mark Caulfield 2011). This pilot study was devised in 2012, and methods for the screening and recruitment of patients were based on the recruitment methods used in the Symplicity HTN-2 trial (Esler, Krum et al. 2010), to try to establish a dataset consistent with contemporaneous clinical research. A clearly defined patient cohort is vital in clinical studies to facilitate the appropriate interpretation of outcome data, and importantly, all RDN procedures at our centre were performed by the same, experienced operator to establish procedural consistency. Extended autonomic patient profiling was performed in study participants prior to RDN, but it should be emphasised that these autonomic data, and data on renal dysfunction beyond an exclusion of eGFR (estimated glomerular filtration rate) <45 mL/min/1.73m^2, were not used to screen or select patients for the trial.

In this pilot study, we had originally planned to recruit twenty patients with treatment resistant hypertension. Recruitment was extended to thirty patients at the time of the ethical amendment to include the procedural efficacy, on-table testing, sub-study (October 2013). The methods and results from screening and recruitment for the study are detailed below.

5.1.2 Methods

Recruitment to the Renal Denervation for Resistant Hypertension Study took place between March 2012 and December 2016. The inclusion and exclusion criteria for the study are summarised in Box 5.1.

5.1.2.1 Pre-screening

Pre-screening of patients took place at the specialist Hypertension Clinic, at the Bristol Heart Institute, led by Dr Angus Nightingale. I was also an active physician in the Hypertension Clinic for a full afternoon per week, participating directly in the diagnosis and management of resistant hypertension, and pre-screening for this pilot study. Patients attending the clinic for the treatment of resistant hypertension, who provisionally met the study and inclusion and exclusion criteria, were approached for discussion about the study, and if the patients were interested in participating in the

study, they were invited for formal screening. Patients were consented for the study prior to formal screening and recruited into the study at the time of their renal denervation procedure.

Standard assessment in the Hypertension Clinic included:

- Clinical review
  - History of course of hypertension
  - History and examination for symptoms and signs of secondary hypertension
  - History and examination for symptoms and signs of target organ damage
  - Social history and review of lifestyle factors contributing to hypertension
- Medication review
  - Optimisation of antihypertensive drug regimen based on current evidence-based practice, including NICE guidelines and trial data (e.g. PATHWAY-2) (National Clinical Guideline 2011, Williams, MacDonald et al. 2015).
  - Optimisation of antihypertensive drug dosages; titration up to maximum licensed dose where possible, but also including strategies such as divided doses, nocturnal dosing and fractional dosing to minimize adverse drug effects and improve adherence (Antoniou, Saxena et al. 2016).
  - Discussion of medication adherence (urine antihypertensive drug metabolite testing was not available at the time of study design)
- Screening for secondary hypertension
  - Ambulatory and/or home blood pressure monitoring
  - Assessment for obstructive sleep apnoea, including Epworth Score
  - Measurement of renal function and albumin:creatinine ratio
  - Thyroid function tests
  - Aldosterone:renin ratio
  - 24hr urinary cortisol
  - 24hr urinary catecholamines
  - 24hr urinary 5HIAA (5-Hydroxyindoleacetic acid)
- Hypertension Protocol magnetic resonance imaging (MRI) scan (see Section 4.3.4)
  - Full cardiac MRI to assess left ventricular function and volumetrics, and to look for evidence of hypertensive heart disease (including left ventricular hypertrophy) or ischaemic heart disease
  - Imaging of the aorta to exclude aorta coarctation
  - Imaging of the renal arteries and parenchyma to look for evidence of renovascular disease or chronic kidney disease
  - Simple adrenal imaging to screen for adrenal adenoma or hyperplasia.

5.1.2.2 Screening

The study inclusion and exclusion criteria are summarised in Box 5.1. These criteria were based on, and are similar to, the enrolment criteria for Symplicity HTN-2 (Esler, Krum et al. 2010), with the notable differences being a broader office SBP inclusion level of >140 mmHg as opposed to >160 mmHg in Symplicity HTN-2 (to optimise recruitment rates), and the addition of an upper limit on body mass index (BMI) of 35 kg/m^2. Full, written

consent was obtained from patients prior to formal screening for the study. Screening consisted of a clinical history and examination, clinic BP (blood pressure) measurement, and a blood test to assess renal function (eGFR). Before leaving the study visit, patients were fitted with a 24hr ambulatory BP monitoring (ABPM) device. Patients also went home with a device for home BP monitoring (HBPM) with a home medication diary as a surrogate marker for medication adherence.

All participants underwent a hypertension MRI scan (see Section 4.3.4.1 for full protocol). Ethical approval provided for the use of CT angiography to confirm suitable renal artery anatomy for RDN if MRI was contraindicated, however, all patients recruited to the study were able to have MRI. Ethical approval was also obtained for the retrospective use of MRI data from clinical scans performed during the preceding 12 months to establish eligibility for RDN whilst avoiding duplicate imaging.

Of note, screening for the study started before a dedicated Clinical Research Fellow was in post, and before ABPM and HBPM devices dedicated for use in the study were available. Consequently, for the patients recruited in the first few months of the study no formal screening (also baseline) ABPM or HBPM/home diary were performed. All patients had had ABPM and/or HBPM performed in the Hypertension Clinic prior to screening for the exclusion of white-coat hypertension, however, some of this monitoring was distant to enrolment in the study, and therefore cannot be used as baseline data due to interim changes in medication. This breech in the protocol was reported to the local ethics committee and was resolved following the provision of appropriate clinical support and equipment.

Patients meeting the inclusion and exclusion criteria, then underwent baseline autonomic profiling prior to elective renal denervation, as per the study protocol (see Section Figure 4-1).

Inclusion criteria

- Clinic systolic BP >140 mmHg
- Patients on at least three anti-hypertensive medications at maximum tolerated dose
- No evidence of secondary hypertension following thorough clinical assessment
- Age 30-75 years

Exclusion criteria

- BMI >35 kg/m^2
- Pregnancy or anticipation of pregnancy
- Palliative care/chemotherapy
- Expected life expectancy less than 12 months due to other disease
- Renal transplant patients
- Variant anatomy of renal artery (e.g. early renal artery bifurcation, small renal arteries <4mm diameter, short renal arteries <20 mm, aberrant renal artery morphology (Esler, Krum et al. 2010)) which makes the patient unsuitable for renal artery denervation
- Renal function impairment (estimated GFR<45ml/min/1.73m^2)
- Heart failure with reduced ejection fraction
- Severe cardiac valvular disease
- Acute coronary syndrome or unstable angina
- Untreated obstructive sleep apnoea
- Intravenous drug use
- Alcohol intake >28 units/week

Box 5.1. Inclusion and exclusion criteria for the Renal Denervation for Resistant Hypertension Study

BP, blood pressure; CKD, chronic kidney disease; BMI, body mass index; GFR, glomerular filtration rate.

5.1.3 Results

Nineteen patients were recruited into the study during the screening period. This was below the target recruitment of thirty patients. Ten patients were recruited into the procedural efficacy sub-study involving on-table testing of afferent and efferent renal nerve integrity.

5.1.3.1 Pre-screening

For the period of 01/01/2013 through to 31/12/2016, there were 1282 appointments scheduled in the Hypertension Clinic at the Bristol Heart Institute. There were 645 individuals who received at least one appointment during that time, with a range of 1-12 appointments per patient. A full analysis of the pre-screening data including the full clinic demographic, diagnostic and outcome at a has not been possible due to time limitations, however, reasons for exclusion from the study at the stage of pre-screening are summarised in the consort diagram in Figure 5-1.

Data from the first 200 patients in the clinic assessed using the Hypertension Protocol MRI have been collated and were presented at the European Society of Cardiology conference (Rome, 2016), prior to publication (Burchell, Rodrigues et al. 2017). Patients who were potentially eligible for the RDN study were referred for MRI, therefore this dataset provides insight into the pre-screening outcomes. Of the 200 scans analysed, 27% of patients had ≥1 accessory renal artery. Furthermore, 29 patients (14.5%) had potential secondary causes of hypertension identified on MRI. There were 12 patients with adrenal masses/hyperplasia, 10 with renal artery stenoses, 6 with renal abnormalities potentially causing secondary hypertension, 7 with thyroid abnormalities, one individual with aortic coarctation, and one with an enlarged pituitary gland (non-functional) (Burchell, Rodrigues et al. 2017). The findings from this study are summarised in Table 5-1.

Patients with resistant hypertension, who were broadly eligible for RDN and interested in pursuing the procedure, were approached regarding the study, and with consent, attended for formal screening.

5.1.4 Screening

Thirty-three patients were screened for the RDN for resistant hypertension study, with 19 patients recruited. Recruitment took place between 20th March 2012 and 15th December 2016. Reasons for screen failure are summarised in Figure 5-1, however, factors included controlled hypertension (n=3), poor renal function (n=2), unsuitable renal anatomy (n=3), adverse comorbidities (previous aortic dissection, recent myocardial infarction, active rheumatoid arthritis requiring immunomodulation), newly diagnosed Conn's syndrome, BMI >35 and withdrawal of consent (n=1).

Pathology	No. cases	Details
Adrenal mass	12	• 7 lesions were not hormonally active.

		• 1 patient with bilateral phaeochromocytomas and a thyroid nodule diagnosed with MEN2a. • 1 patient had a resolution of their hypertension following treatment with spironolactone. • 3 patients unable to exclude endocrine pathology
Renal artery stenosis	10	Cases reviewed at renal multidisciplinary team meeting: – 2 referred for stenting – 8 for medical management
Renal abnormality	21	6/21 findings may reflect secondary hypertension: – 2 atrophy secondary to RAS – 2 atrophy not related to RAS – 1 polycystic kidney disease. – 1 renal coloboma syndrome (autosomal dominant condition characterised by renal hypodysplasia, optic nerve dysplasia and hypertension) (Schimmenti 2011).
Thyroid abnormality	7	Goitre and nodules; assessed biochemically and referred for further investigation if indicated. 1 case MEN2a see above.
Pituitary enlargement	1	Investigated with pituitary function testing and a pituitary MRI; non-functional.
Aortic coarctation	1	Associated with bicuspid aortic valve and aortopathy. Novel diagnosis, hypertension resolved following endovascular stenting.

Table 5-1. Secondary causes of hypertension in a cohort of 200 consecutive patients from a Specialist Hypertension Clinic, as demonstrated by MRI.
MEN; multiple endocrine neoplasia, RAS; renal artery stenosis. Adapted from Burchell et al. 2017.

5.1.4.1 Study completion and withdrawals

17 participants completed the study with full 12 months follow-up. Of the remaining two patients, one withdrew from the study immediately after the RDN procedure due to problems with hypotensive side-effects post-denervation, and one patient withdrew after 6 months follow-up due to a lack of treatment effect and long travel times to Bristol. The patient who withdrew from the study immediately after the procedure was one of the early participants in the study, with more limited data. For this reason, this patient has not been included in any of the subsequent data analyses.

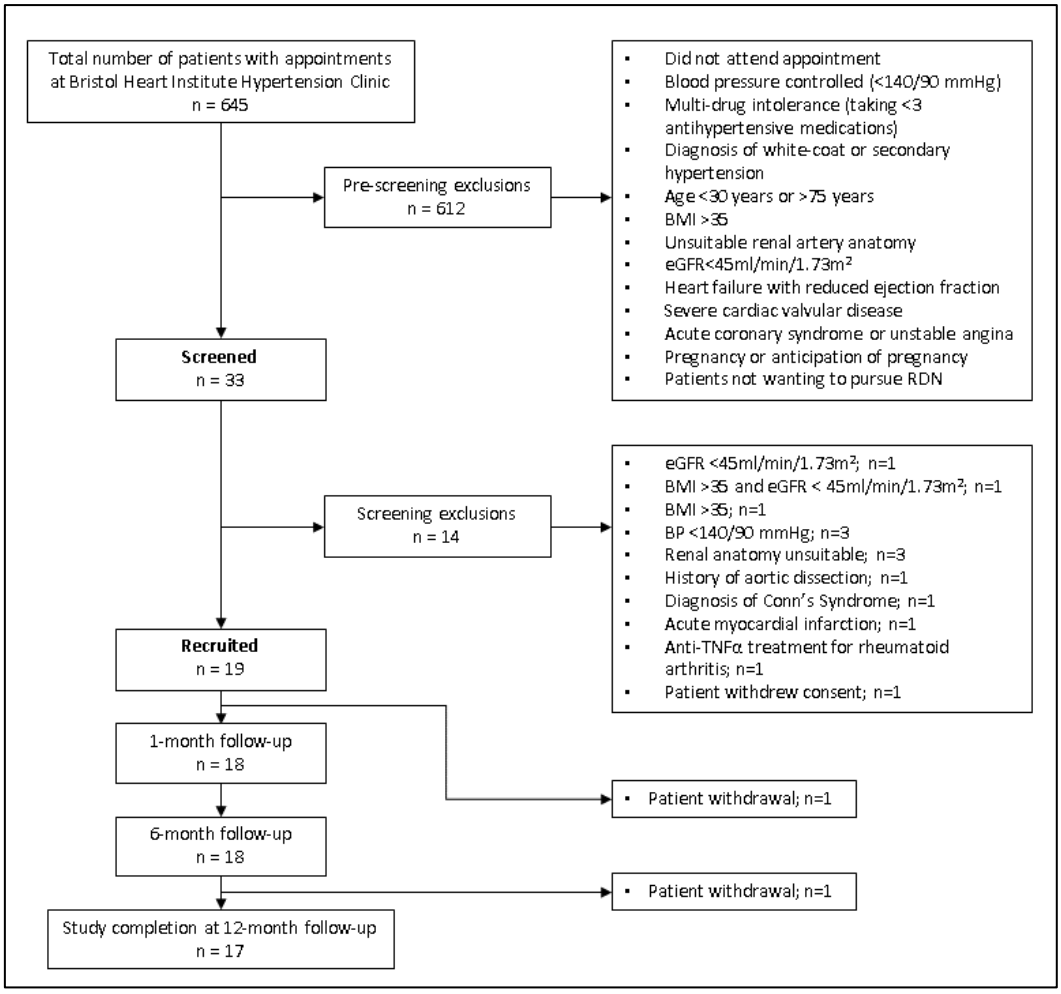


Figure 5-1. Consort diagram illustrating the study recruitment process and patient follow-up.
Pre-screening commenced in January 2012, and recruitment to the study took place between March 2012 and December 2016. BMI, body mass index; eGFR, estimated glomerular filtration rate; RDN, renal denervation; TNFα, tumour necrosis factor alpha.

5.1.4.2 Baseline demographic data

Baseline demographic data for the 18 patients with follow-up to at least 1-month post-RDN are summarised in Table 5-2. 17 patients were of white Caucasian ethnicity, and one patient was of mixed white and Afro-Caribbean ethnicity. Half of the study participants were female; of these, three had a history of hypertension in pregnancy or pre-eclampsia and four were post-menopausal. Of the patients with diabetes mellitus, one individual had type 1 diabetes, one had type 2 diabetes requiring insulin, and a third had borderline, diet-controlled type 2 diabetes. In addition, one patient had had a thyroidectomy due to thyrotoxicosis with thyroid function stable on replacement therapy, one patient had had an aortic valve replacement and coronary artery bypass grafting 11 years prior to enrolment in the study due to childhood rheumatic fever, one patient was registered as partially-sighted, and one patient who had previous venous thromboembolic disease, had undergone unilateral carotid body resection as part of a

pilot study investigating carotid body excision as a treatment for hypertension >2 years before entering this study.

5.1.4.2.1 Baseline antihypertensive medications

The 18 participants analysed were prescribed a mean of 5.2 ± 1.8 antihypertensive medications (range 3-8), equivalent to 4.0 ± 2.4 whole dose equivalents (WDE; range 0.7-9.5, see Table 5-3). The WDE is the proportion of the maximum licenced dose of a medication that has been prescribed (Antoniou, Saxena et al. 2016). The maximum dose is that specified by the British National Formulary (Publications 2017). For example, a patient prescribed 5 mg of Ramipril, which has a maximum dose of 10 mg, would be receiving 0.5 WDE. 14/18 patients were prescribed a diuretic; all patients had been trialled on diuretic medication, but in four patients this medication was discontinued due to intolerance. Ten patients were taking an aldosterone antagonist, reflecting optimal management of resistant hypertension based on data from the PATHWAY -2 study (Williams, MacDonald et al. 2015). Of the other eight patients not taking an aldosterone antagonist, five had documented intolerance to spironolactone, whilst three had not had a documented trial of spironolactone (although it should be noted that these patients underwent RDN prior to the publication of the PATHWAY-2 results).

All patients were asked about whether they took all of their antihypertensive medication at the baseline clinical assessments, and all participants confirmed adherence, however, medications are documented as having been prescribed since adherence was not formally assessed in this study. Home blood pressure monitoring, with a surrogate medication diary was completed by 7/18 patients. The mean home BP was 184 ± 21/95 ± 16 mmHg amongst these 7 patients; all patients had a home SBP of >135 mmHg (range 159-224/ 68-116 mmHg). All patients who completed a HBPM diary self-reported medication adherence, but the amount of information provided in the diaries was variable.

	Participants (n=18)
Office SBP (mmHg)	192 ± 21
Office DBP (mmHg)	105 ± 23
24hr SBP (n=10, mmHg)	166 ± 13
24hr DBP (n=10, mmHg)	94 ± 12
Day SBP (n=13, mmHg)	171 ± 14
Day DBP (n=13, mmHg)	99 ± 14
Night SBP (n=12, mmHg)	156 ± 18
Night DBP (n=12, mmHg)	86 ± 12
Age (years)	55 ± 11
Male gender	9
BMI (kg/m^2)	29 ± 3
eGFR (mL/min/1.73m^2)	74 ± 11
Albumin:creatinine ratio (mg/mmol)	8 ± 13

Left ventricular hypertrophy	14
Past medical history	
Ischaemic heart disease	5
Hypercholesterolaemia	4
Diabetes mellitus	3
TIA/CVE	5
Peripheral vascular disease	1
Asthma	2
Treated OSA	1

Table 5-2. Baseline demographic data for study participants.
All data shown are for n=18 study participants unless otherwise specified (ABPM data). Data are mean ± standard deviation, or (if no units) n. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; eGFR, estimated glomerular filtration rate; TIA, transient ischaemic attack; CVE, cerebrovascular event; OSA, obstructive sleep apnoea.

	Participants (n=18)
Antihypertensive medications	5.2 ± 1.8
Antihypertensive medication classes	4.8 ± 1.8
Whole dose equivalent	4.0 ± 2.4
Drug classes	
ACEi/ARB/RI	18
Calcium channel blocker	12
Diuretic	14
Thiazide/Thiazide-like diuretic	11
Loop diuretic	2
Aldosterone antagonist	10
β blocker	11
α-1 blocker	11
Centrally acting sympatholytic*	7
Vasodilator	4

Table 5-3. Antihypertensive medication prescribed at baseline.
Data are mean ± standard deviation. Whole dose equivalent is the sum of the proportions of the maximum licensed doses for each of the medications prescribed. *All patients prescribed moxonidine. ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; RI, direct renin inhibitor.

5.1.5 Discussion

More than 600 patients were pre-screened via the Bristol Heart Institute Hypertension Clinic to identify 19 patients eligible for this study, giving a recruitment rate of approximately 3%. We were unable to achieve the target recruitment of 30 participants due to the challenge of identifying patients with true, treatment resistant hypertension, suitable renal artery anatomy, who met the stringent inclusion and exclusion criteria, and who were happy to participate in a study involving a novel interventional therapy. There was a high enrolment rate following formal screening (19/33; 56%), but this reflects the fact that most of the patients who attended for screening were well known to the clinical members of the research group due to parallel commitments in the Hypertension Clinic.

Symplicity HTN-2 recruited 106 participants out of the 190 patients screened (56%) (Esler, Krum et al. 2010), and in Symplicity HTN-3 535/1441 (37%) patients were enrolled (Bhatt, Kandzari et al. 2014). This yield must reflect a significant degree of pre-screening, as it is considerably highly than the estimated prevalence of resistant hypertension, which is likely to be closer to 10% of the hypertensive population (Calhoun, Jones et al. 2008, de la Sierra, Segura et al. 2011, Egan, Zhao et al. 2011, Persell 2011, Barochiner, Alfie et al. 2013). Indeed, in this context, a recruitment rate of 3%, following a thorough assessment for pseudo-resistance, optimisation of medications and application of the study inclusion and exclusion criteria (e.g. BMI and renal function cut-offs and renal artery anatomical criteria), is perhaps not unexpected, and the enrolment target of 30 patients was always going to have been difficult to achieve in a single-centre study. Any future studies in this population should either plan to recruit across multiple sites, or to adopt a broader range of hypertensive patients, perhaps including those with multi-drug intolerance, who continue to have a significant unmet clinical need. Ideally, an armoury of treatment options is needed, such that those with treatment resistant, or drug intolerant, hypertension can be offered alternative therapy if they are unsuitable for RDN. This was a pilot study, and as such, no power calculation was performed, so it is not possible to quantify the impact of sub-optimal participant numbers.

Despite slightly broader hypertension inclusion criteria, our study population was more severely hypertensive than the RDN cohort in Symplicity HTN-2 (mean office BP 192/105 mmHg vs 178/97 mmHg, respectively), and had a greater proportion of females (50% vs 35%, respectively), but was similar in other respects (mean values: age 55 vs 58 yrs, BMI 31 vs 29 kg/m^2, eGFR 77 vs 74 mL/min/1.73m^2, both 5.2 antihypertensive medication) (Esler, Krum et al. 2010). Our participants more closely reflected the RDN treatment group in Symplicity HTN-3; office BP 192/105 vs 180/97 mmHg, females 50% vs 40%, age 55 vs 58 yrs, BMI 29 vs 34 kg/m^2, antihypertensive medications 5.2 vs 5.1 (mean values, study patients vs Symplicity HTN-3 RDN treatment group patients, respectively) (Bhatt, Kandzari et al. 2014). The similarity between our patient cohort's baseline demographics and those of the Symplicity studies reflects the similar inclusion and exclusion criteria and is helpful when considering the transposition of findings from this study onto larger populations of patients with severe drug resistant hypertension.

One of the major limitations of the present study, is the lack of formal ABPM baseline data in all study participants. 13/18 patients had had a formal ABPM at screening/baseline, although the data were not complete in all cases due to extreme high BP readings resulting in multiple error readings, or an inability to tolerate the ABPM

device for the full 24-hour period. If it was acceptable to the patients, sub-optimal ABPM was repeated, but this was not always possible. As explained above, screening for the study started before ABPM and HBPM devices dedicated for use in the study were available. Consequently, for the patients recruited in the first few months of the study no formal screening or baseline ABPM was performed. All patients had had ABPM and/or HBPM performed in the Hypertension Clinic prior to screening for the exclusion of white-coat hypertension, but this does mean that the baseline dataset for the study was incomplete.

A major criticism of recruitment in this study, which is also applicable to recruitment in Symplicity HTN-2&3 (Esler, Krum et al. 2010, Bhatt, Kandzari et al. 2014), is the absence of data confirming medication adherence. Levels of non-adherence may be as high as 50% in the hypertensive population (Jung, Gechter et al. 2013), and were even higher in the SYMPATHY renal denervation study which identified poor adherence, or non-adherence, in 80% of patients in a retrospective sub-study of urinary drug metabolites (de Jager, de Beus et al. 2017). Furthermore, given that non-adherence may be partial and variable over time, undocumented changes in antihypertensive medications during the study could have represented a significant confounding factor. The blood pressure and medication diaries issued to the participants were completed to a varying extent, with some patients just documenting the times that medications were taken, whilst others provided a comprehensive list of times, drugs and dosages. This could be improved by adapting the format of the diary and providing better patient information. There is now, however, a wide consensus that the use of a medication diary as a surrogate marker for mediation concordance, as used in Symplicity HTN-2, and upon which the design of this study was based, is an inadequate tool for the assessment of medication adherence in studies of interventional treatments for hypertension (Fadl Elmula, Hoffmann et al. 2013, Hameed, Pucci et al. 2015, Schmieder, Ott et al. 2016). In light of this, we have obtained ethical approval to obtain consent from our participants for the retrospective measurement of drug metabolites in stored and frozen urine samples. This will not only inform the interpretation of any changes in BP over the course of the study but will also give further insight into levels of adherence amongst study participants in trials of interventional therapies for hypertension.

In Symplicity HTN-2, of the 190 patients assessed for eligibility, 84 were excluded; 36 (19%) due a systolic BP <160 mmHg after a two week of self-reported confirmation of medication compliance, 30 (16%) due to ineligible renal artery anatomy, 10 (5%) who declined to participate, and 8 (4%) due to other unspecified factors (Esler, Krum et al. 2010). Symplicity HTN-3 included ABPM as an additional part of the screening process and required a systolic BP of <160 mmHg at two separate screening visits, two weeks apart, on stable medication (Bhatt, Kandzari et al. 2014). In Symplicity HTN-3, of the 1441 patients assessed for eligibility, 463 patients (31%) were found to have an office SBP of <160 mmHg, 41 (3%) were not on maximal medical therapy, 178 (12%) had unsuitable renal artery anatomy, 50 (3%) had an eGFR of <45 mL/min/1.73m^2, 53 (4%) had significant comorbid condition, recent hypertensive crises or orthostatic hypotension, and 43 (3%) had a 24hr SBP of <135 mmHg on ABPM (the remaining patients excluded refused, withdrew or were unable to consent) (Bhatt, Kandzari et al. 2014, Waksman, Bakris et al. 2017). In both studies, the main reason for exclusion from the study was a controlled BP following the use of a medication diary, and repeated BP assessment. This emphasises the importance of robust baseline BP assessment, and

confirmation of a stable medication regime, prior to enrolment, to limit the impact of regression to the mean and Hawthorne effects on BP outcomes.

Several smaller studies have also looked at the process of screening patients for RDN in more detail. Verloop et al. used a standardised, stepwise approach (including assessment for secondary hypertension, ABPM and then renal artery imaging) to screen 181 patients who were referred for RDN (Verloop, Vink et al. 2013). 121 patients (67%) were excluded from RDN; 23 patients (19%) had an office SBP <160 mmHg, 26 patients (22%) showed a white-coat effect, 14 (12%) had a novel diagnosis of secondary hypertension, and 9 patients had ineligible renal artery anatomy. Patel et al. also used a stepwise approach to screen 34 patients for renal denervation, at the end of which, only 5 patients were eligible for the procedure, four of whom refused consent (Patel, Gupta et al. 2016). Looking more closely at the reasons for screen failure in the latter study, four patients (12%) had insufficiently high BP, two patients (6%) had white-coat effect, 29% (10 patients) were non-adherent, 6 patients (18%) were on suboptimal treatment, 3 patients (9%) had primary aldosteronism, and of the seven patients meeting the clinical inclusion and exclusion criteria, two were excluded due to inappropriate renal artery anatomy. Ultimately these studies, along with published interim data from the CardioNomics group combined with data from St Bartholomew's Hospital which reported a 10% yield from screening (33/321 patients) (Burchell, Chan et al. 2016), highlight the importance of rigorous assessment for causes of pseudo-resistant hypertension if a firm baseline patient demographic is to be established. The process is vital to confirm a treatment effect of RDN beyond potential causes of confounding, however, it is important to remember that any results obtained from studies using these highly defined resistant hypertension cohorts are only directly applicable to other similar patients who would meet the study criteria, rather than the general hypertensive population. As established above, identifying patients who meet these stringent recruitment criteria is challenging, and is a major limitation of the current evidence base for RDN

The SPYRAL HTN studies aimed to address the criticisms over recruitment which have been levied against the earlier Symplicity trials through a range of measures (Kandzari, Kario et al. 2016, Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018). ABPM was mandated in the screening process to exclude white-coat hypertension, and medication adherence to a standardised regime in the ON-MED study (thiazide-type diuretic, dihydropyridine calcium-channel blocker, and ACEi or ARB), or the absence of medication in the OFF-MED study was confirmed at screening and during follow-up by plasma and urine high-pressure liquid chromatography–tandem mass spectroscopy. Furthermore, the hypertensive population enrolled was more closely defined, with the exclusion of patients with isolated systolic hypertension (office DBP<90 mmHg), and patients were required to be on a stable antihypertensive regime, that did not include an aldosterone antagonist, for at least 6 weeks (ON-MED study). Interim data are now available; for the SPYRAL HTN OFF-MED study 353 patients have been formally screened for the study in order to recruit an initial 80 patients for randomisation. This reflects a 23% recruitment rate from randomisation, reflecting a more moderate level of hypertension at baseline, and the exclusion of those with isolated systolic hypertension (SYPRAL HTN BP criteria defined as office SBP ≥150 mmHg and <180 mmHg, office DBP ≥90 mmHg, and a mean 24-h ambulatory SBP ≥140 mmHg and <170 mmHg) (Townsend, Mahfoud et al. 2017). The investigators have aimed to try to limit any Hawthorne effect

through the removal of inter- and intra-participant differences in medication prescription and adherence as a confounding factor, and through the use of ABPM as the primary outcome measure, which due to its automated nature and repeated measures is felt to be a less variable measure, and less vulnerable to bias and regression to the mean (Townsend, Mahfoud et al. 2017).

This study was designed to echo the format of Symplicity HTN-2, and before the publication and critique of Symplicity HTN-3, and as discussed, there are several limitations in the study screening process. The screening methods and criteria for this study cannot be altered retrospectively, but these issues will be taken into consideration when interpreting the study data and outcomes.

5.2 Clinical Outcomes: Safety, blood pressure and target organ damage

5.2.1 Introduction

Whilst this study aimed to identify markers to aid patient selection and assess procedural success in renal denervation, it is important to add to the existing safety and clinical outcome data in the field which was presented in Section 2.3.6. These factors will ultimately contribute to the successful largescale roll-out of RDN into clinical practice. In this section, I will present the safety data for our cohort, along with blood pressure outcomes (including reference to medication changes and adherence) and look at the impact of RDN on target organ damage including excretory renal function, albuminuria, aortic function, and left ventricular function, hypertrophy and fibrosis. The relationship between the level of target organ damage and any change in blood pressure following RDN will also been assessed.

5.2.2 Methods

5.2.2.1 Safety

Procedural safety measures included monitoring of renal function using estimated glomerular filtration rate (eGFR) at 1, 3, 6 and 12-month follow-up, and assessment for renal artery stenosis by follow-up magnetic resonance angiography (MRA) at 6 months post-RDN (see protocol in Section 4.3.4). Adverse events (AEs) occurring between enrolment and study completion were documented according to the University Hospitals Bristol NHS Foundation Trust Research and Innovation Research Safety Reporting protocol (University Hospitals Bristol NHS Foundation Trust 2017). Adverse events were classified as mild (easily tolerated by the patient, not interfering with daily activities), moderate (sufficiently discomforting to interfere with normal daily activities) or severe (preventing normal daily activities), with severe adverse events including those resulting in death, that were life-threatening, that required hospitalisation or prolonged existing hospitalisation, that resulted in persistent incapacity or disability, or that resulted in a congenital abnormality or birth defect. Adverse events were classified as expected or unexpected based on previously reported adverse events in the literature, with a statement about likely causality. All serious adverse events were reported to the study sponsor (University Hospitals Bristol NHS Foundation Trust) and were declared in the annual report to the Research Ethics Committee.

5.2.2.2 Blood pressure outcome data

Office and ambulatory blood pressure was assessed at baseline and 1, 3, 6 and 12-months post-RDN according to the methods outlined in Section 4.3.2.

5.2.2.3 Heart rate

Mean resting heart rate (HR) was calculated from the 3-lead ECG recording obtained during a 5-10-minute period of quiet, semi-supine, rest.

5.2.2.4 Total peripheral resistance

An estimate of total peripheral resistance (TPR) was calculated as follows. TPR = MAP - RAP/CO, where MAP was the mean arterial pressure obtained from office BP recordings; RAP was the right atrial pressure which for the purposes of this calculation was assumed to be negligible, and CO was the cardiac output calculated from the cardiac magnetic resonance imaging (CMR) volumetric data (CO = HR x stroke volume). The CMR protocol is described in Section 4.3.4.

5.2.2.5 Prescribed medications

Prescribed medications were documented at each study visit. Whole dose equivalents were calculated for each antihypertensive medication to facilitate the comparison between types and classes of drug (see Section 5.1.4.2.1). The patients were also asked by the reviewing clinician to confirm adherence to medication. A formal medication diary was performed as part of screening but was not repeated at follow-up visits.

5.2.2.6 Assessment of target organ damage

Renal function was assessed through the measurement of eGFR from venous blood samples, quantified using the MDRD (Modification of Diet in Renal Disease) Study equation (Levey and Inker 2017). Renal end-organ damage was also quantified through the measurement of the urinary albumin:creatinine ratio.

All patients underwent comprehensive CMR at baseline and then again 6 months after renal denervation. This included quantification of left ventricular (LV) mass and volumes, quantification of left ventricular hypertrophy (LVH) and remodelling, assessment of left ventricular fibrosis using late gadolinium enhancement and functional quantification with measurement of ejection fraction and LV strain parameters.

The standard CMR protocol is described in Section 4.3.4. Further to this, strain analysis assessed the degree of regional myocardial deformation and its timing during the cardiac cycle. Strain imaging was performed with voxel-tracking post-processing software (Tissue Tracking, CMR42, Circle Cardiovascular Imaging Inc., Calgary, Canada) on two-chamber, four-chamber, and short-axis stack SSFP cine images by defining the endocardial and epicardial borders (excluding papillary muscles and trabeculae) and the mitral valve annular plane at end-diastole (Bistoquet, Oshinski et al. 2008, Rodrigues, Amadu et al. 2016). Strain is expressed as the percentage of shortening or lengthening of a small element of myocardium in relation to its original length (Gotte, Germans et al. 2006). Strain analysis uses specialist software to track individual voxels within the myocardium. It is then calculated based on an established algorithm (Bistoquet, Oshinski et al. 2008, Rodrigues, Amadu et al. 2016). The different directions of myocardial strain are illustrated in Figure 5-2. Global longitudinal strain was the averaged strain from four-chamber and two-chamber analyses. Circumferential and radial strain were calculated as

mean values of mid-myocardial segments from the short-axis cine two-dimensional strain model (Rodrigues, Amadu et al. 2016).

In a sub-set of 7 patients, LV interstitial fibrosis was assessed using T1 mapping. Myocardial T1 mapping was performed using the modified look-locker inversion-recovery (MOLLI) sequence [flip angle: 358, minimum time to inversion (TI): 100 ms, TI increment: 80 ms, time delay: 150 ms, heart beat acquisition scheme: 5-(3)-3] (Messroghli, Greiser et al. 2007). Regions of interest were drawn within the mid-septum on short-axis, motion-corrected native T1 maps and transposed onto corresponding 15-min post-contrast maps for analysis (Rodrigues, Amadu et al. 2016). T1 analysis was performed with Argus software (Siemens, Erlangen, Germany), as previously described (Pica, Sado et al. 2014). The T1 values were the mean of all pixels within the region of interest. The extracellular volume fraction (ECV) was calculated as: ECV = $(\Delta R1_{myocardium}/\Delta R1_{blood\text{-}pool}) \times (1 - haematocrit)$, where ΔR1 = (1/post-contrast T1 - 1/native T1). Myocardial cell volume (MCV) fraction was defined as 1 - ECV and multiplied by indexed myocardial volume (indexed LVM divided by 1.05 g/mL, the myocardial specific gravity). Indexed interstitial volume (IV) was defined as ECV × indexed myocardial volume (Rodrigues, Amadu et al. 2016).

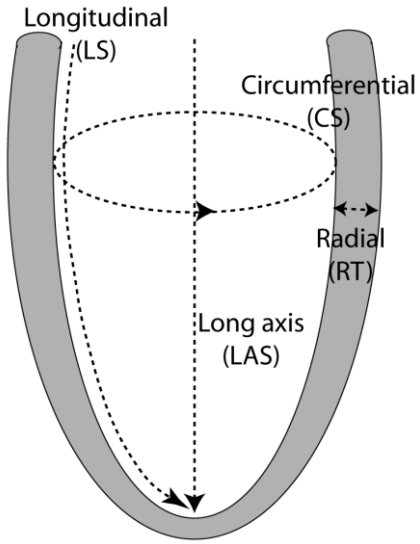


Figure 5-2. Dimensions of myocardial strain.
Myocardial strain quantifies the degree of myocardial deformation (percent change) in three different dimensions; longitudinal strain (LS), radial strain (RS), and circumferential strain (CS). These directions of strain are pictured in reference to the long axis (LAS) of the left ventricle in the figure above (Cheng-Baron, Nelson et al. 2011).

Ascending aortic stiffness was also assessed from the CMR data as previously described (Groenink, de Roos et al. 2001, Rodrigues, Amadu et al. 2015, Rodrigues, Amadu et al. 2016). Ascending aortic compliance = ΔA/ΔP where: ΔA (mm^2) was defined A_{syst}–A_{diast}. A_{syst} and A_{diast} were measured from cine images perpendicular to the ascending aorta at the level of the right pulmonary artery, and are the areas of the ascending aorta measured at end-systole and end-diastole respectively. Ascending aortic distensibility

was estimated as follows: distensibility = $\Delta A/(A_{diast} \times \Delta P)$. ΔP (in mmHg) is the pulse pressure estimated from SBP minus DBP. Measurements were acquired by an experienced blinded CMR reader (Dr J. Rodrigues). Excellent reproducibility of these measures has previously been reported (Groenink, de Roos et al. 2001).

Changes in the recorded measures of target organ damage were correlated against the patients' change in office systolic BP at 6 months post-RDN.

5.2.3 Results

5.2.3.1 Safety

5.2.3.1.1 Renal parameters.

Assessing data from all 18 participants, there was a significant reduction in eGFR at the primary end-point, 6 months post-RDN (74 ± 3 ml/min/1.73m^2 vs 71 ± 3 ml/min/1.73m^2 at 0 and 6 months, respectively, p=0.048, see Figure 5-3). Over the full course of the study there was also a significant reduction in eGFR (data carried forward, p=0.01) with a significant difference between the mean eGFR at baseline and that at 12 months (74 ± 3 ml/min/1.73m^2 vs 67 ± 4 ml/min/1.73m^2 respectively, p<0.05; see Figure 5-4). When the eGFR data are categorised by BP response to RDN (responders: ≥10 mmHg fall in office SBP at 6 months), there was no significant change in eGFR during the study amongst the responders, however, the non-responders showed a significant reduction in eGFR (data carried forward; see Figure 5-4). At 6 months post-RDN, the change in office SBP correlated with the change in eGFR, with those with no change in SBP, or an increase in SBP following RDN showing a reduction in eGFR (see Figure 5-5). There was no significant difference in baseline eGFR between responders and non-responders (74 ± 4 ml/min/1.73m2 vs 72 ± 3 ml/min/1.73m2 respectively, p=0.73).

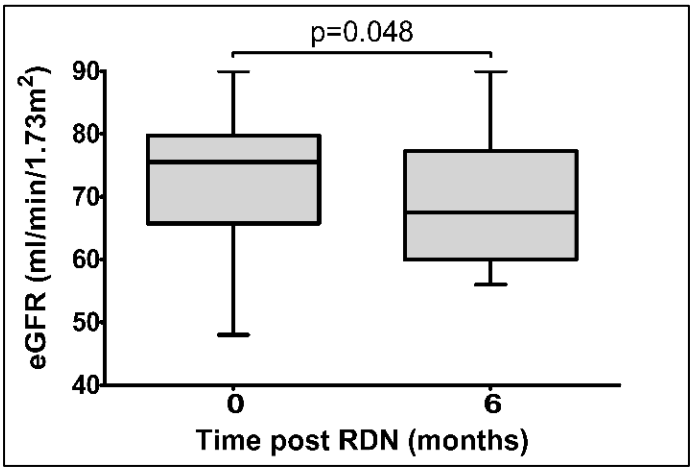


Figure 5-3. Significant reduction in estimated glomerular filtration rate (eGFR) at 6 months after renal denervation.
Data for all 18 participants, analysed by paired Student's t-test

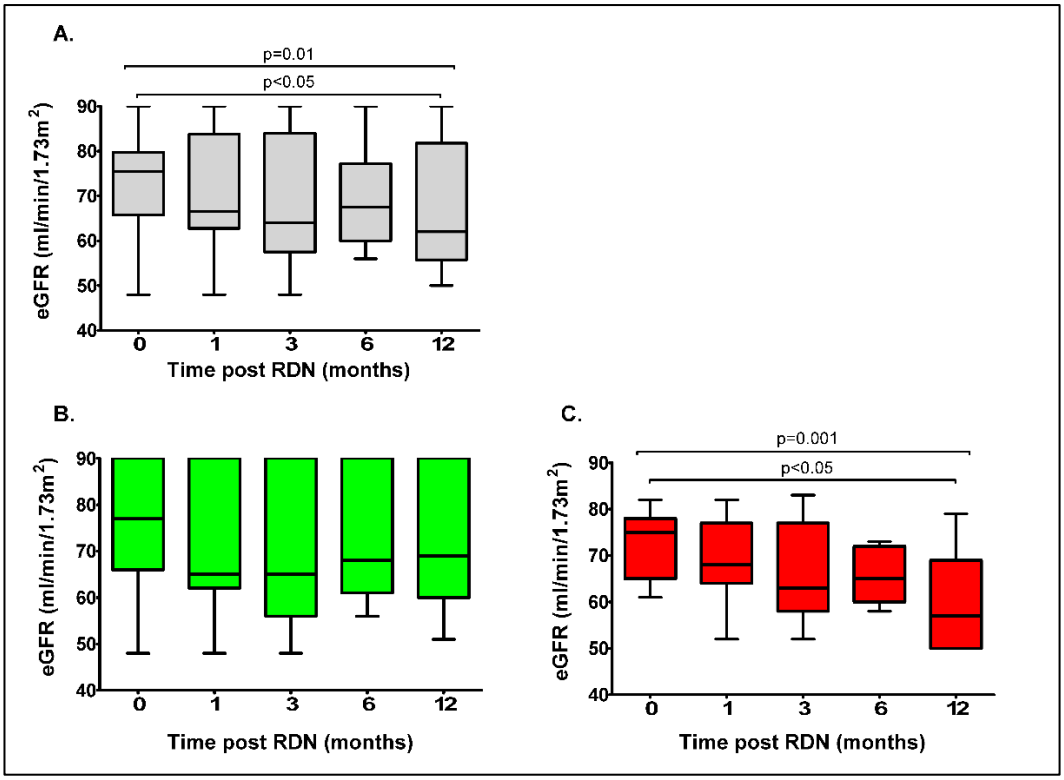


Figure 5-4. Change in renal function as assess by estimated glomerular filtration rate (eGFR) following renal denervation (RDN).
Data for all 18 participants (A), RDN responders (B, n=11) and RDN non-responders (C, n=7) is shown. The upper p value reflects the results from a Friedman Test, the lower p value is from Dunn's Multiple Comparison Test (significant data shown). Please note, the maximum eGFR reported by our laboratory is >90 ml/min/1.73m^2, limiting assessment of change in eGFR in those with normal excretory function.

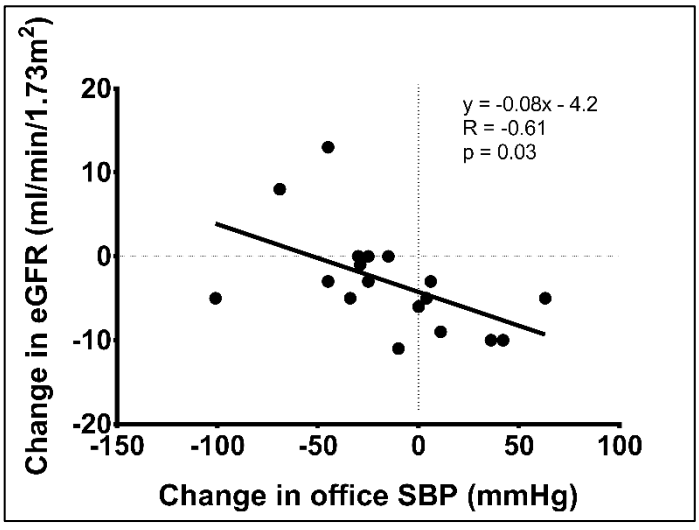


Figure 5-5. Correlation between the change in office systolic blood pressure (SBP) and the change in estimated glomerular filtration rate (eGFR) at 6 months post-RDN.
Follow-up MRI scans were performed on average 207 ± 10 days post procedure (range 164-318 days). Renal artery stenosis was not detected on MR angiography in any of the 18 study participants.

5.2.3.1.2 Adverse Events

There were 29 adverse events during the study, of these, 21 were classified as serious adverse events, affecting 13 patients. Seven of the serious adverse events occurred during the screening period in patients not ultimately recruited to the study, however, since these events occurred following study consent but prior to formal exclusion and withdrawal, they have been reported to the study sponsor. The serious adverse events are summarised in Table 5-4, and it should be noted that of the non-serious events, syncope was documented in two patients following the procedure, resulting in adjustments in antihypertensive medications.

Patient	Event	Comment
Events occurring prior to RDN		
A	Unstable angina requiring bypass grafting	Met exclusion criteria
A	Unstable angina requiring percutaneous coronary intervention	Met exclusion criteria
2	Chest pain and palpitations	Diagnosed with non-cardiac pain following investigation
B	Rectal bleeding	Met other exclusion criteria
C	Worsening severe renal impairment	Met exclusion criteria
D	Haematuria and urinary retention resulting in hospitalization	Not recruited
D	Fall with head injury secondary to hypotension	Labile BP due to autonomic failure, not recruited.
Events occurring after RDN		
2	Fractured foot following mechanical fall	No associated postural hypotensive symptoms, felt unlikely to be related to RDN.
4	Fractured fibula following mechanical fall	
8	Possible transient ischaemic attack	Normal CT brain
10	Haematoma at femoral artery access site post-procedure	Related to RDN, recognized complication of angiography.
12	Pyelonephritis requiring intravenous antibiotics	Remote to procedure, not felt to be related
13	Cerebrovascular event	Remote to procedure, not unexpected in patient with sustained hypertension following RDN.
15	Admission following RDN prolonged by 2 days due to hypotension	Related to RDN, but not unexpected
17	Admission following RDN prolonged by 3 days due to hypotension with acute kidney injury	Sequalae fully resolved. Related to RDN, but not unexpected.

17	5 further admissions due to severe symptomatic hypertension, including novel diagnosis of cerebellar infarction	Not unexpected, in patient with severe hypertension.
18	Unprovoked pulmonary embolus	Remote to procedure, not felt to be related to RDN.

Table 5-4. Summary of serious adverse events occurring during the study.
Patients enrolled in the study are indicated by their numerical study identification number. Patients screened for, but not enrolled in, the study are designed by a letter patient identifier.

5.2.3.2 Blood pressure outcomes

5.2.3.2.1 Office blood pressure

11/18 (61%) patients responded to RDN with an office BP reduction of ≥10 mmHg at 6 months post-RDN, however, the individual responses to RDN were highly variable (see Figure 5-6), and there was no significant difference in the principle study BP endpoint, between baseline office systolic BP (oSBP) and 6-month oSBP (192 ± 5 mmHg vs 177 ± 7 mmHg, n=18, p=0.10). The mean change in office blood pressure was -11 ± 8/-4 ± 5 mmHg (n=17, p=0.20/0.67), -13 ± 9/-4 ± 5 mmHg (n=15, p=0.18/0.93), -16 ± 9/-2 ± 6 mmHg (n=18, p=0.10/0.56) and -26 ± 8/-11 ± 5 mmHg (n=17, p=0.005/0.04) at 1, 3, 6 and 12 months following RDN, respectively (p values for change in office BP are for Student's t-test versus zero baseline). Whilst there was no significant change in oSBP by the primary 6-month outcome measure, office systolic and diastolic BP had significantly reduced amongst the 17 participants who attended 12-month follow-up. There were also significant changes in mean oSBP and mean office diastolic BP (oDBP) as assessed by 1-way ANOVA with data carried forward (see Figure 5-7).

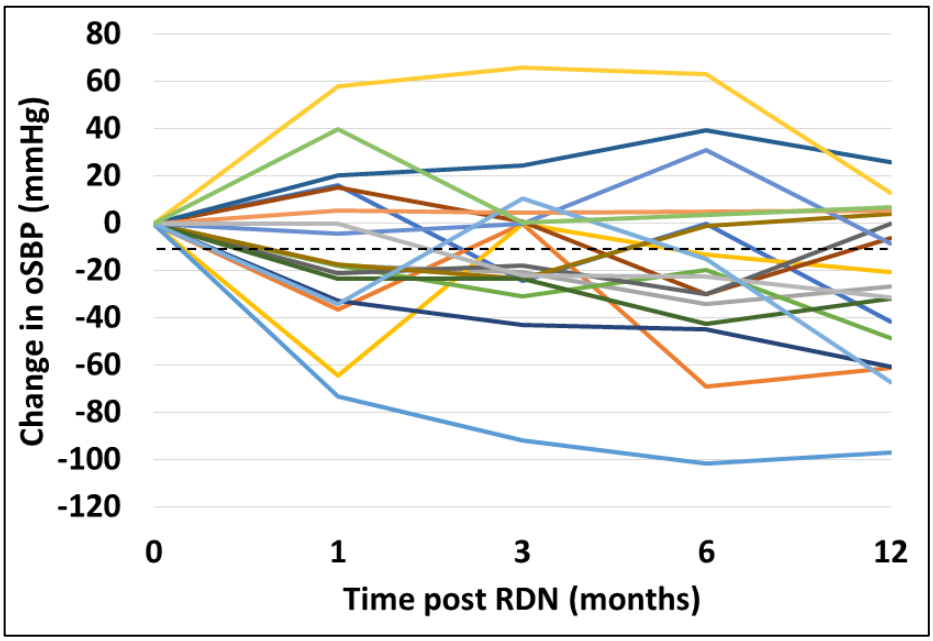


Figure 5-6. Change in office systolic blood pressure (oSBP) following renal denervation (RDN), shown for individual study participants.

Dashed line indicates 10mmHg reduction in oSBP; responders to renal denervation were defined as those participants with a ≥10 mmHg reduction in oSBP at 6 months post-RDN.

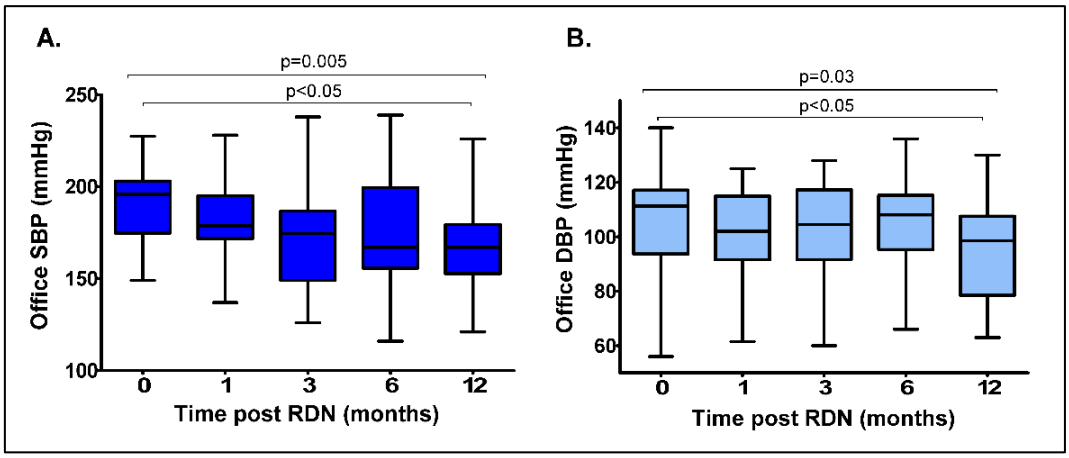


Figure 5-7. Mean office systolic (A.) and diastolic (B.) blood pressure at baseline and then 1, 3, 6 and 12 months follow-up post-RDN.
There were significant changes in office systolic and diastolic BP by 1-way ANOVA (mean carried forward for missing data) across the study visits (upper p value), with a significant reduction in oSBP and oDBP seen at 12 months after denervation as compared with baseline (Bonferroni between group comparison).

5.2.3.2.2 Ambulatory blood pressure

24hr ambulatory blood pressure monitoring (ABPM) data were obtained in 10/18 participants at baseline. In one of these participants, ABPM data were successfully obtained at baseline, but not at any of the study follow-up visits, and therefore outcome 24hr ABPM data can only be presented for 9/18 patients. This subset of patients includes 4 RDN responders and 5 RDN non-responders based on 6-month oSBP results. The mean reduction in 24hr ABPM in the subset of patients with available data was -2 ± 4/-1 ± 2 mmHg (n=7, p=0.56/0.87), -5 ± 7/0 ± 5 mmHg (n=6, p=0.50/0.67), -7 ± 4/-1 ± 3 mmHg (n=8, p=0.12/0.83) and -5 ± 7/-3 ± 5 mmHg (n=8, p=0.55/0.64) at 1, 3, 6 and 12 months, respectively (p values for change in office BP are for Student's t-test versus zero baseline). Analysis of the absolute 24hr ABPM data showed no significant change in systolic or diastolic BP across the follow-up period post-RDN (repeated measures 1-way ANOVA with data carried forward; n= 9, p=0.47/0.82, see Figure 5-8).

Daytime ABPM data were obtained in 13/18 participants (6/13 of these were RDN responders, individual data shown in Figure 5-9). The mean change in mean daytime BP in this subset of patients with available data was -3 ± 7/0 ± 5 mmHg (n=11, p=0.64/0.96), -9 ± 9/-3 ± 6 mmHg (n=9, p=0.38/0.62), -8 ± 6/-3 ± 4 mmHg (n=12, p=0.37/0.53) and -11 ± 8/-7 ± 5 mmHg (n=10, p=0.28/0.26) at 1, 3, 6 and 12 months, respectively (p values for change in office BP are for Student's t-test versus zero baseline). Analysis of the absolute

daytime ABPM data showed no significant change in systolic or diastolic BP across the follow-up period post-RDN (repeated measures 1-way ANOVA; n= 13, p=0.14/0.13, see Figure 5-10). The was no change in mean night-time ABPM over the course of the study (n=11, p=0.19).

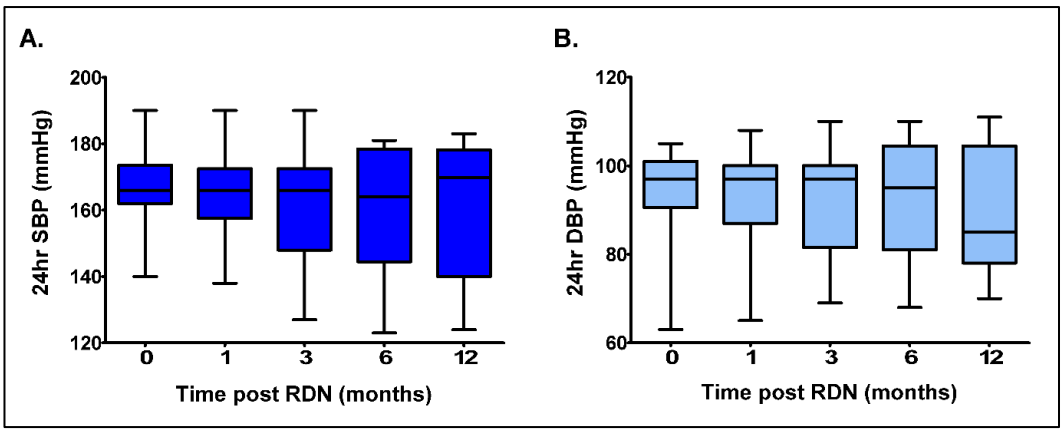


Figure 5-8. Mean 24hr ambulatory systolic (A.) and diastolic (B.) blood pressure at baseline and then 1, 3, 6 and 12 months follow-up post-RDN.
There was no significant change in 24hr systolic or diastolic BP by 1-way ANOVA across the study visits (n= 9, p=0.47/0.82).

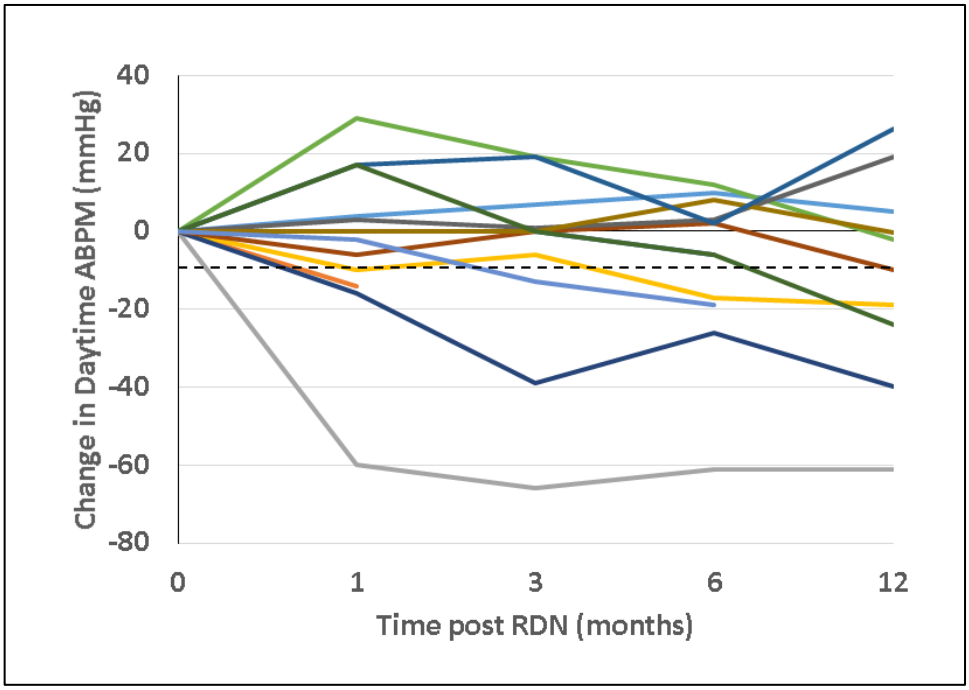


Figure 5-9. Change in daytime ambulatory systolic blood pressure (ABPM) following renal denervation (RDN).
Data shown for individual patients (n=13). Dashed line shows ABPM reduction of 5 mmHg which is commonly used as the cut-off to define a clinical response to RDN as assessed by ABPM (Mahfoud, Ukena et al. 2013).

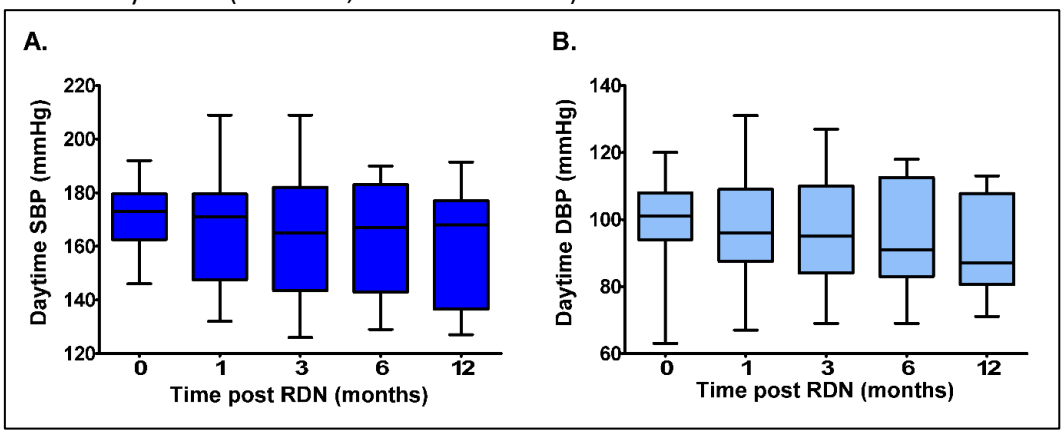


Figure 5-10. Mean daytime ambulatory systolic (A.) and diastolic (B.) blood pressure at baseline and then 1, 3, 6 and 12 months follow-up post-RDN.
There was no significant change in daytime systolic or diastolic BP by 1-way ANOVA across the study visits (n= 13, p=0.14/0.13).

Data for daytime ABPM, which had the greatest number of available data points (13 participants), was analysed by RDN response group. When considering the outcome at 6 months post-RDN analysed by paired Student's t-test, there was no significant change in mean daytime systolic or diastolic BP amongst in either responders (n=5/13, no 6-month data for one responder) or non-responders (n=7/13). When data were analysed by repeated measures ANOVA with data carried forward, RDN responders (n=6/13) had a significant reduction in daytime ABPM (daytime SBP and DBP both p=0.02), with significant differences between both systolic and diastolic baseline daytime ABPM versus daytime ABPM at 12 months by Dunn's multiple comparison test (173 ± 7 mmHg vs 146 ± 8 mmHg, $p<0.05$ and 105 ± 4 mmHg vs 87 ± 4 mmHg, $p<0.05$, respectively). There was no significant change in daytime ABPM amongst RDN non-responders (n=7/13) over the course of the study when assessed by repeated-measures ANOVA.

5.2.3.3 Heart rate

The was no significant change in mean resting heart rate at 6 months post-RDN (66.5 ± 2.1 bpm vs 66.5 ± 3.3 bpm, n=18, p=0.99, bpm: beats per minute) Furthermore, there was no significant change in HR over the course of the study when analysed by repeated measures ANOVA (data carried forward); heart rate 66.5 ± 2.1 bpm, 64.6 ± 2.3 bpm. 63.6 ± 2.2 bpm, 66.5 ± 3.3 bpm, and 63.6 ± 2.2 bpm at 0, 1, 3, 6 and 12 months post-RDN, respectively (p=0.47). There was no significant correlation between baseline oSBP and baseline resting HR (R=0.35, p=0.15), and no correlation between the change in oSBP at 6-months post-RDN and the change in mean HR 6 months post-RDN (R=-0.02, p=0.92). When analysed by RDN BP-response group, there was no significant change in HR amongst either RDN responders (n=11) or non-responders (n=7) either by paired t-test at baseline versus 6 months (n=11, p=0.83 and n=7, p=0.52, respectively) or by

repeated-measures ANOVA across all time points (data carried forward; p=0.24 and p=0.33 for responders and non-responders, respectively).

5.2.3.4 Total peripheral resistance

There was no significant difference between estimated TPR at baseline versus 6 months post-RDN (20.2 ± 1.3 mmHg/l/min vs 20.1 ± 1.3 mmHg/l/min, n=18, p=0.96). At baseline, there was no correlation between oSBP and TPR (R=0.27, p=0.28). There was a strong correlation between the change in oSBP at 6-months post RDN and the change in TPR at the same timepoint (R=0.73, p=0.0005, see Figure 5-11). RDN responders tended towards a reduction in TPR at 6 months, although this was not significant (Δ -2.2 ± 1.7 mmHg/l/min, n=11, p=0.22), whereas RDN non-responders had a significant increase in TPR following RDN (Δ 3.3 ± 0.7 mmHg/l/min, n=7, p=0.004).

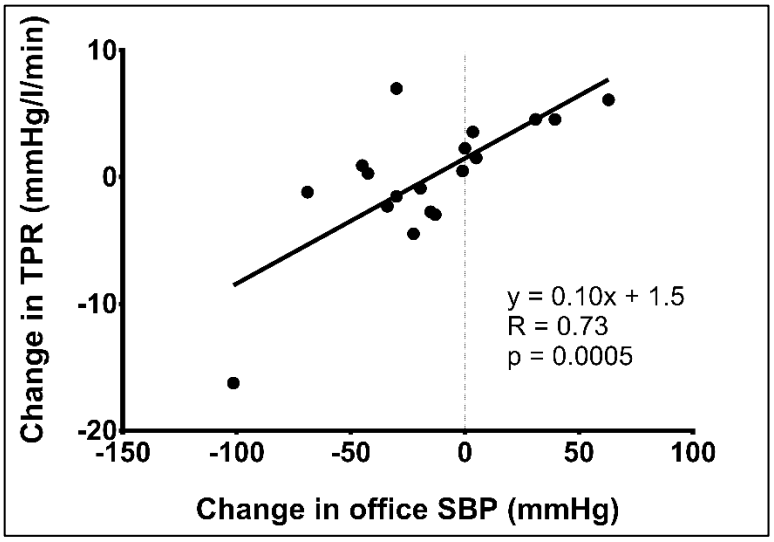


Figure 5-11. Correlation between the change in office systolic blood pressure (SBP) and the change in total peripheral vascular resistance (TPR) at 6 months after renal denervation.
Data for all eighteen study participants. Data shown are for Pearson's correlation coefficient (R), with significance taken as $p<0.05$; a reduction in SBP following RDN was associated with a reduction in TPR.

5.2.3.5 Medications

The primary intention of the study was for medications to remain unchanged following baseline assessment, until 12-month follow-up was complete. 16/18 patients had changes to their prescribed medication over the course of the study, this included decreases in medication in patients with symptomatic hypotension, increases in medication in participants with ongoing severe hypertension, and one participant who discontinued all their medication following the RDN procedure against medical advice. The details of the specific medications prescribed, and changes made over the course of the study are provided in Appendix 1; these data are summarised in Table 5-5.

	Time post RDN (months)					
	0	1	3	6	12	P
No. antihypertensive drugs	5.2 ± 0.4	4.2 ± 0.4	4.4 ± 0.5	4.4 ± 0.5	4.9 ± 0.5	0.02
No. antihypertensive drug classes	4.8 ± 0.4	3.9 ± 0.4	4.1 ± 0.4	4.2 ± 0.5	4.6 ± 0.4	0.02
Whole dose equivalents	4.0 ± 0.6	3.1 ± 0.5	3.3 ± 0.5	3.3 ± 0.5	3.7 ± 0.5	0.05

Table 5-5. Mean prescribed medications by total number of antihypertensive drugs, total number of antihypertensive drug classes and whole dose equivalents.
The whole dose equivalent is the sum of the proportions of the maximum licensed dose prescribed of the patient's medications. The p value is for the repeated measures 1-way ANOVA for all 18 patients.

5.2.3.6 Target organ damage

5.2.3.6.1 Renal function

Data for renal function as estimated by changes in eGFR over the course of the study were reported in Section 5.2.3.1.1. Microalbuminuria is a prognostic marker of cardiovascular risk (Viazzi, Cappadona et al. 2016). Baseline albumin: creatinine ratio (ACR) was 8.0 ± 3.6 mg/mmol (range 0.5 – 51.6 mg/mmol, n=14). Eleven participants had follow-up ACR data at 6 and/or 12 months post-RDN. There was no change in ACR between during follow-up in this subset of patients 8.0 ± 4.5 mg/mmol, 9.5 ± 4.3 mg/mmol and 10.8 ± 4.0 mg/mmol at 0, 6 and 12 months, respectively (repeated measures Friedman test p=0.47). There was also no significant change in ACR when analysed by response to RDN (responders p=0.96, non-responders p=0.25).

5.2.3.6.2 Cardiac structure and function

Cardiac MRI (CMR) data were obtained in all 18 patients before and after RDN. The baseline and follow-up CMR data are summarised in Table 5-6.

Parameter	Pre-RDN	Post-RDN	P
Volumetrics			
LVEF (%)	66 ± 2	66 ± 2	0.79
LV mass (g)	177 ± 13	159 ± 11	0.01
Indexed LV mass (g/m^2)	90 ± 6	80 ± 5	0.01
EDV (ml)	162 ± 8	155 ± 8	0.21
Indexed EDV (ml/m^2)	82 ± 3	79 ± 4	0.23
ESV (ml)	56 ± 5	54 ± 5	0.67
Indexed ESV (ml/m^2)	28 ± 2	27 ± 2	0.62
SV (ml)	106 ± 5	101 ± 5	0.19
Indexed SV (ml/m^2)	53 ± 2	51 ± 2	0.28
Strain			
Peak radial strain (%)	32 ± 2	35 ± 2	0.13

Peak circumferential strain (%)	-18 ± 1	-20 ± 1	0.15
Peak longitudinal strain (%)	-18 ± 1	-19 ± 1	0.14
Peak systolic radial strain rate (%/sec)	196 ± 17	218 ± 13	0.16
Peak systolic circumferential strain rate (%/sec)	-105 ± 7	-110 ± 4	0.40
Peak systolic longitudinal strain rate (%/sec)	-98 ± 6	-95 ± 4	0.55
Peak diastolic radial strain rate (%/sec)	-201 ± 20	-181 ± 13	0.34
Peak diastolic circumferential strain rate (%/sec)	110 ± 11	89 ± 5	0.06
Peak diastolic longitudinal strain rate (%/sec)	115 ± 12	95 ± 4	0.08

Table 5-6. Left ventricular volumetric and strain parameters as assessed by cardiac MRI, before and 6-months after renal denervation (RDN).
Data for all 18 participants, indexed to body surface area and expressed as mean ± SEM. Difference between measures pre- and post-RDN were assessed using a paired Student's T test, significance taken as $p<0.05$. LVEF; left ventricular ejection fraction, LV, left ventricular, EDV; end diastolic volume, ESV; end systolic volume, SV; stroke volume.

Following RDN, there was a significant reduction in LVM and LVM index (see Table 5-6 and Figure 5-12), whilst other volumetric parameters remained unchanged. The change in office SBP at 6-months post-RDN correlated with the changes in LVM ($R=0.62$, $p=0.006$), indexed LVM ($R=0.56$, $p=0.02$), peak radial strain ($R=-0.53$, $p=0.02$), and peak circumferential strain ($R=0.54$, $p=0.02$) (see Figure 5-13).

At baseline 14/18 (78%) patients had left ventricular hypertrophy (LVH), 0/18 patient had left ventricular remodelling and 4/18 patients had normal range LV mass and morphology. Following RDN, 11/18 (61%) patients had LVH, 2/18 patients were defined as having LV remodelling, and 5/18 patients had normal range LV mass and M/V parameters. When assessed via late gadolinium enhancement 2 patients had evidence of previous myocardial infarction at baseline, with one of these individuals also having evidence of LV fibrosis, after denervation, a separate participant was reported to have regression of raised LVM, but increased LV fibrosis.

When considering LV systolic function, at baseline, 16/18 participants had a LV ejection fraction (LVEF) of >55%; 2/18 patients had an LVEF of between 50% and 55%, no patients had an LVEF of <50%. Following RDN, LVEF was >55% in all participants, bar one individual, who had responded to RDN with a BP reduction, but had a decrease in LVEF from 71% to 47% (it should be noted that this individual had discontinued their cardiac medication against medical advice). Despite this pattern for a normalisation of LVEF, there was no change in mean LVEF following RDN (see Table 5-6). The strain data may indicate a pattern for an increase in peak strain in all dimensions after denervation, but none of these changes attained significance. There was a borderline decrease in peak diastolic circumferential and longitudinal strain rate (see Table 5-6).

There was no correlation between baseline office SBP and any of the CMR volumetric or strain parameters at baseline (all $p>0.05$).

When considering the changes in the CMR parameters by response group, RDN responders had borderline reductions in LVM and LVM index (n=11, -22 ± 8 g and -11 ± 4 g/m^2 respectively, both $p=0.05$), but no significant changes in any of the other volumetric or strain parameters. Non-responders also had a reduction in indexed LVM (n=7, -9 ± 2 g/m^2, $p=0.03$), but no other changes in the CMR parameters were observed.

There were no significant differences in any of the CMR indices listed in Table 5-6 at baseline, or on repeat imaging after RDN, between BP responders and non-responders (all p>0.05).

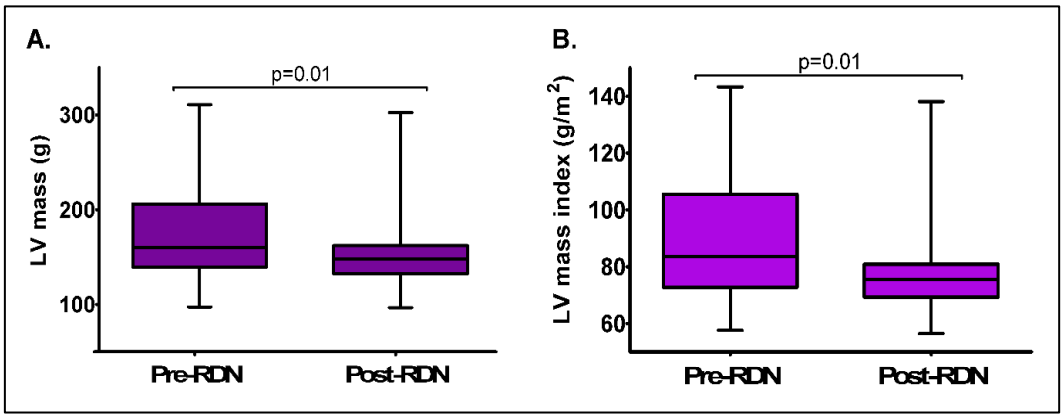


Figure 5-12. Left ventricular mass (A) and left ventricular mass index (B), before and 6 months after renal denervation (RDN).
Data for all 18 participants. P value refers to paired Student's T test.

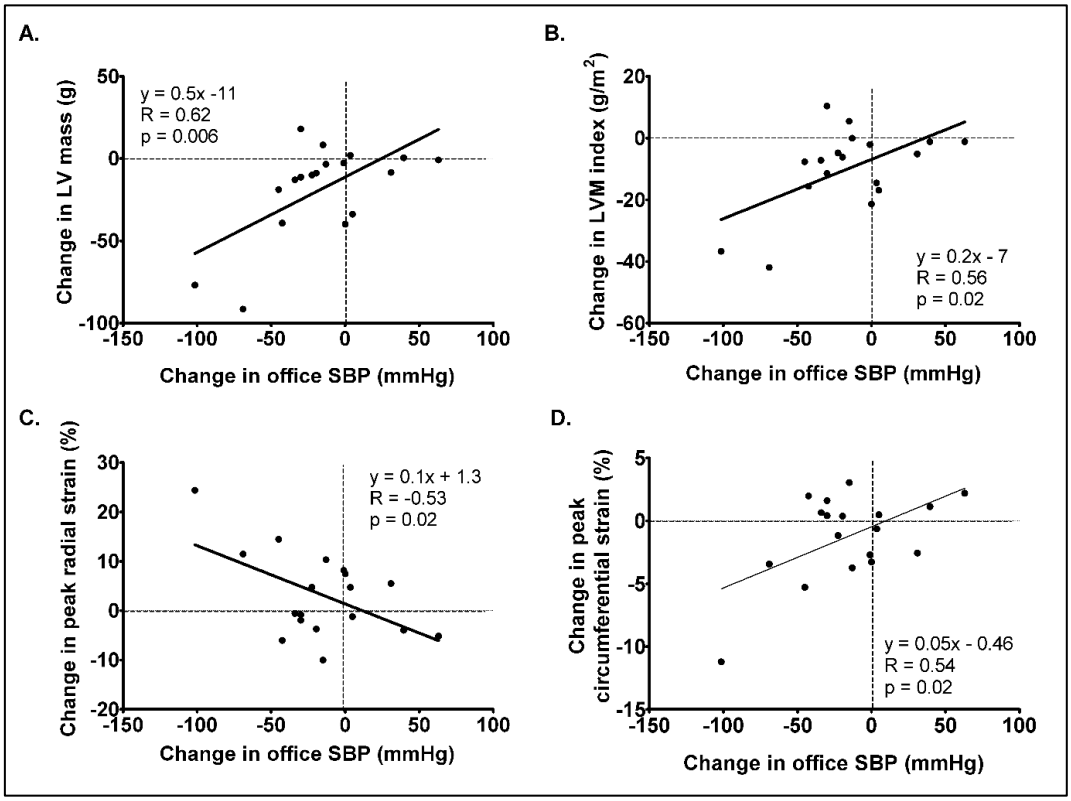


Figure 5-13. Correlations between the change in office systolic blood pressure (SBP) following renal denervation, and the changes in A. Left ventricular mass, B. Left ventricular mass index, C. Peak radial strain and D. Peak circumferential strain, at 6 months after renal denervation.

Data for all eighteen study participants. Data shown are for Pearson's correlation coefficient (R), with significance taken as $p<0.05$. A reduction in SBP was associated with an improvement (reduction) in LV mass and LV mass index, and improvements in peak radial (increased thickening) and peak circumferential (increased shortening) strain.

In a subset of 7 participants, T1 mapping was performed to better assess the effect of RDN on LV interstitial fibrosis. The T1 mapping data are summarised in Table 5-7; none of the T1 parameters listed correlated with office SBP at baseline (all $p>0.05$). There was a significant reduction in indexed interstitial volume, with a trend towards a reduction indexed myocardial cell volume, and an overall trend towards a reduction in the ECV (see Table 5-7). There were no correlations between changes in any of the T1 mapping parameters and the change in office SBP at 6 months post-RDN (all $p>0.05$) and given the small number of participants in this sub study, it is not appropriate to analyse by RDN response group.

Parameter	Pre-RDN	Post-RDN	P
Extracellular volume fraction	0.27 ± 0.01	0.26 ± 0.01	0.06
Interstitial volume (ml)	49.6 ± 9.3	44.6 ± 8.0	0.08
Indexed interstitial volume (ml/m^2)	25.1 ± 3.9	22.2 ± 3.5	0.04
Myocardial cell volume (ml)	132.8 ± 21.4	126.1 ± 19.6	0.19
Indexed myocardial cell volume (ml/m^2)	67.6 ± 8.7	63.0 ± 8.2	0.07

Table 5-7. T1 mapping parameters before and after renal denervation (RDN).
Data for 7 participants with T1 mapping data. P value refers to paired Student's T Test.

5.2.3.6.3 Aortic distensibility

Data on aortic compliance and distensibility were available for 15/18 patients at baseline; all of these patients had follow-up data on aortic function at 6 months post-RDN. There was no significant correlation between baseline office SBP and either baseline aortic compliance or baseline aortic distensibility (R=0.03, p=0.93 and R=0.16, p=0.57, respectively). There was no significant change in aortic compliance or distensibility following RDN, even once analysed by RDN BP-response subgroup (see Table 5-8). In keeping with the latter result, there was no correlation between the change in office SBP at 6-months post RDN and the change in either aortic compliance or distensibility following the procedure (R=-0.43, p=0.11 and R=-0.36, p=0.18, respectively), although the trend in these data may suggest that a reduction in SBP following RDN is associated with an increase in aortic distensibility.

Parameter	Pre-RDN	Post-RDN	P
Aortic compliance (mm^2/mmHg)	1.31 ± 0.22	1.63 ± 0.24	0.11
Responders	*1.48 ± 0.27*	*1.88 ± 0.32*	*0.18*
Non-responders	*1.06 ± 0.40*	*1.26 ± 0.35*	*0.46*
Aortic distensibility (mm^2/mmHg x10^3)	1.59 ± 0.28	1.86 ± 0.24	0.31

Responders	*1.82 ± 0.38*	*2.13 ± 0.29*	*0.46*
Non-responders	*1.24 ± 0.38*	*1.45 ± 0.38*	*0.52*

Table 5-8. Aortic compliance and distensibility before and after renal denervation (RDN).
Data are presented for the 15 patients with suitable aortic imaging available at baseline, and also by subgroup analyse according to BP response to RDN (in italics). 9/15 were classified as responders (office SBP reduction of ≥10 mmHg at 6 months post RDN) and 6/15 were non-responders. P value refers to paired Student's T Test.

5.2.4 Discussion

5.2.4.1 Safety

Our data are consistent with those from large commercial studies and registry data (previously reviewed in Section 2.3.6) in demonstrating an acceptable safety profile for RDN (Esler, Krum et al. 2010, Bhatt, Kandzari et al. 2014, Vogel, Kirchberger et al. 2014, Sharp, Davies et al. 2016, Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018). In this cohort, there was one complication directly related to the procedure (femoral haematoma), but reassuringly, none of the patients developed de novo renal artery stenosis following renal endovascular ablation. Two patients had prolonged admissions (>24hrs) following RDN due to symptomatic hypotension. The hypotension may well be a result of the acute haemodynamic changes following disruption of the renal nerves since both patients also responded to RDN at 6 months, however, it also possible that inpatient admission for assessment and titration of antihypertensive medications unmasked poor medication adherence. All other adverse events were either not attributable and/or remote to the intervention or were not unexpected in patients with severe resistant hypertension (see Table 5-4).

The ENCOReD meta-analysis reported no adverse effect of RDN on renal function (Fadl Elmula, Jin et al. 2015). Amongst our study cohort, there was a significant reduction in eGFR over the course of the study. However, on closer analysis, this decline in renal function was limited to those patients with sustained (or worsening) severe hypertension who failed to respond to RDN, and was not seen amongst RDN BP - responders, and thus does not suggest that effective RDN induces a greater rate of renal dysfunction. Our data emphasise uncontrolled hypertension as a risk factor for chronic kidney disease (CKD) (Ott, Mahfoud et al. 2015), and in keeping with this, renal denervation has previously been shown to preserve renal function in patients with CKD and resistant hypertension (Ott, Mahfoud et al. 2015).

5.2.4.2 Blood pressure outcomes

61% of study participants responded to RDN with a ≥10 mmHg reduction in office SBP at 6 months post denervation. The study failed to achieve one of its principle outcome measures in that there was no significant reduction in office SBP at 6 months after RDN. However, there was a significant change in office SBP across the follow-up period (ANOVA p=0.005), reaching significance at 12 months (see Figure 5-7).

What is clear from Figure 5-6, is that the individual BP responses were highly variable, with some patients developing a clinically significant (≥10 mmHg, n=11) reduction in office SBP 6 months post-RDN, whilst in others little BP effect (n=4) or an increase in BP (n=3) was observed. The mean reduction in office SBP at 6 months in this study was not of the magnitude seen in Symplicity HTN-1&2 (-22mmHg and -32 mmHg, respectively), although our 12-month data are more comparable, and the response rate of 61% was much lower than the 87% and 84% response rates reported in these studies (Krum, Schlaich et al. 2009, Esler, Krum et al. 2010). The ALSTER and Heidelberg registries also report better response rates of 76% (n=93) and 73% (n=63) respectively (Kaiser, Beister et al. 2014, Vogel, Kirchberger et al. 2014). Our data are more reflective of the success rates seen in other European studies (Zuern, Eick et al. 2013, Hameed, Pucci et al. 2015, Rohla, Nahler et al. 2015), and data from the UK Renal Denervation Affiliation (office BP reduction of 22/9 mmHg ($p<0.001$) in a cohort of 246 patients from 16 centres) (Sharp, Hameed et al. 2015). Likewise, Persu et al. report a response rate of 59.6% in their meta-analysis of 10 European expert RDN centres (Persu, Jin et al. 2014), and the Global Symplicity Register of 998 patients gives a response rate of 67% (Bohm, Mahfoud et al. 2015).

The SPYRAL HTN-OFF MED study reflects a slightly different patient population (more moderate hypertension, off medication) with study investigators reporting a preliminary 3-month office SBP reduction of -10.0 mmHg (-15.1 to -4.9; p=0·0004) (Townsend, Mahfoud et al. 2017). Longer term outcomes are awaited for this study, however, the data presented do demonstrate the individual variability in the BP response to RDN which must be taken into account when counselling and consenting patients prior to the procedure (Townsend, Mahfoud et al. 2017). The reduction in office SBP reported in the preliminary results of the SYPRAL HTN-ON MED study was of an even smaller magnitude (-6·6 mmHg (-12·4 to -0·9; p=0·03)at 6 months) (Kandzari, Bohm et al. 2018). These findings support our clinical impression that whilst renal denervation is effective in some patients, it is not a panacea for all patients with poorly controlled hypertension.

There was no significant change in mean 24hr BP or daytime BP either across the study follow-up period, or at any specific time point versus baseline when analysed for participants with available data (see Figure 5-8 and Figure 5-10). The subset of 13 patients with available daytime ABPM data included 6 RDN responders, and 7 RDN non-responders, and on sub-group analysis, there were significant reductions in daytime systolic and diastolic BP amongst RDN-responders, not seen in the non-responder group. This may not seem surprising, since the responders, by definition had had a reduction in office SBP following RDN, but it is an important observation, since ABPM data represent a more robust outcome measure (Mahfoud, Ukena et al. 2013), and may more accurately predict mortality and morbidity than office BP measurement (Dolan, Stanton et al. 2005, Dolan, Stanton et al. 2009). Incomplete ABPM data is a major limitation of this study, which must rely on oBP outcomes as the primary outcome measure. This is due, in part, to a lack of dedicated ABPM devices in the early part of the study, however, the major factor limiting data was patients' inability to tolerate the ABPM device. Patients with extreme high BP require high cuff inflation pressures, which can be uncomfortable for patients. We also experienced problems with multiple error readings due to extreme high BP, and for one patient with very severe hypertension (office BP often >250 mmHg) accurate ABPM data could not be obtained.

5.2.4.3 Heart rate and total peripheral resistance

It is interesting to note that there was no significant reduction in mean resting heart rate or estimated TPR following RDN in this cohort. If RDN is mediated by a reduction in sympathetic tone, then a reduction in heart rate (which is in part under sympathetic control) might have been anticipated. Interestingly, the effect of RDN on heart rate is not reported in Symplicity HTN 1,2 or 3 ((Krum, Schlaich et al. 2009, Esler, Krum et al. 2010, Bhatt, Kandzari et al. 2014), however, in a cohort of 35 patients in which sympathetic nerve activity was assessed following RDN, Herring et al. report no change in resting HR (Hering, Marusic et al. 2014). It may be that there are too many confounding variables impacting this acutely responsive physiological parameter to see an outcome 'signal' above the noise. HR will also be confounded by negatively chronotropic medications such as β blockers or non-dihydropyridine calcium channel blockers, which may blunt the effect of any change in SNA.

Estimated TPR did not change significantly over the course of the study and was not correlated with office SBP at baseline. There was, however, a strong correlation between the change in oSBP at 6-months post RDN and the change in TPR at the same timepoint (R=0.73, p=0.0005, Figure 5-11), with RDN-responders trending towards a reduction in TPR and RDN non-responders showing an increase in TPR. This observation may purely reflect the fact that TPR is dependent on SBP as a moderator of MAP, but does suggest that the increase in TPR secondary to hypertension may not be fully reversible in this time frame. The limitations of this calculated estimate of TPR must be emphasised; it is derived from measures of office SBP and CMR cardiac output, which were not measured simultaneously, and assumes negligible right atrial pressure. The estimate of TPR does provide a useful variable when interpreting other physiological changes following RDN presented in subsequent sections of this manuscript.

5.2.4.4 Medication changes

The primary intention of the study was for medications to remain unchanged following baseline assessment, until 12-month follow-up was complete. Despite this, the majority of patients (16/18) had changes to their prescribed medication over the course of the study. There were a variety of reasons for this, the most significant of which was the reduction in the number of antihypertensive drugs in the initial post-procedural period due to symptomatic hypotension. These medications had largely been reintroduced by the time these patients reached 6-month follow-up. Medications were also changed in response to patient reported side-effects, altered by other medical teams, and in one case a participant discontinued all their medication following the RDN procedure against medical advice. These changes in prescribed medication are clearly a major limitation for this study and present a confounding factor when interpreting outcome and physiological data. A more rigorous and formally structured medication regime, such as that used in DENERHTN or the SPYRAL-ON MED studies would have helped to address these issues (Azizi, Sapoval et al. 2015, Kandzari, Kario et al. 2016).

A stepped, standardise prescribing regimen goes part way to addressing confounding due to changes in medication, and would help in interpreting physiological data since the patients would be on the same classes of drugs, however, it does not confirm medication adherence, and cannot fully address a Hawthorne effect due to increased

medication adherence following study recruitment. Participants need to be on a stable, well-tolerated, medication regime, with confirmed adherence (for example with testing of urinary drug metabolites over the course of 4 months) prior to RDN, to establish a robust baseline. A structured plan must also be in place for a standardised step-down in medication in the event of symptomatic hypotension, and effective communication between different teams involved in the patients' care is required to prevent changes in medication outside the context of the study, unless clearly clinically mandated.

5.2.4.5 Target organ damage

Hypertension is an established risk factor for, and predictor of, cardiovascular mortality and morbidity (Dolan, Stanton et al. 2005, Dolan, Stanton et al. 2009). A reduction in blood pressure is therefore an established primary outcome measure in studies of novel antihypertensive therapies, however, any reduction in BP must ultimately translate into a reduction in hypertensive target organ damage, cardiovascular disease, and potentially even mortality. A study of this scale is clearly not able to provide data on mortality outcomes, but a reduction in target organ damage following RDN would further support the use of this invasive treatment in patients with refractory hypertension. From a mechanistic perspective it is also interesting to consider whether any improvement in target organ damage is correlated with a reduction in hypertension and/or a reduction in sympathetic tone.

There was an increase in eGFR amongst those patients who failed to respond to RDN and had sustained hypertension. This decline in eGFR was not seen in the patients who responded to RDN, and whilst this does not demonstrate an improvement in renal function following denervation, it does support the reno-protective effects of optimising BP control in patients with resistant hypertension, or may suggest that the disruption of renal sympathetic innervation (particularly in the context of sympathoexcitation as observed in hypertension (Yamada, Miyajima et al. 1989, Malpas 2010)) may protect the kidney independent of the effect of reduced BP.

There was a significant reduction in LVM following renal denervation, notably, three subjects no longer met the LVM threshold for left ventricular hypertrophy 6 months after the procedure. In the sub group of 7 patients with T1 mapping data, there was a reduction interstitial fibrosis. The T1 mapping data show a pattern for a decrease in both interstitial volume and myocardial cell volume (by indexed parameters), consistent with the reduction in LV mass seen in the full cohort. However, overall there was a trend towards a reduction in extracellular volume fraction, suggesting that reduced interstitial fibrosis predominates over a reduction in the hypertrophy of cardiac myocytes, in the remodelling and mass reduction of the LV following RDN.

Evidence for improvement in LV function following RDN is less robust in this cohort. There was no improvement in LVEF, with only a trend in the data for an increase in peak strain in each dimension. Conversely, there was a borderline decrease in peak diastolic circumferential and longitudinal strain rate (see Table 5-6). Additional data from a larger study population are required to clarify the effect of RDN on LV function, but these pilot data would support the need for further investigation.

A reduction in LVM index and an improvement in LV function have been demonstrated previously, but have not been related to reductions in BP, following RDN (Brandt, Mahfoud et al. 2012, Doltra, Messroghli et al. 2014, Mahfoud, Urban et al. 2014, Schirmer, Sayed et al. 2014, McLellan, Schlaich et al. 2015, Tsioufis, Papademetriou et al. 2015, Kiuchi, Mion et al. 2016, Tsioufis, Papademetriou et al. 2016). Brandt et al. reported a reduction in LVM index as assessed by echocardiography, from 112 ± 34 g/m^2 to 95 ± 30 g/m^2 (n=48, p<0.001), as well as improvements in mitral valve lateral E/E', isovolumic relaxation time and ejection fraction (baseline: 63 ± 8% vs. 70 ± 12%, p<0.001) at six months post-RDN, these changes were not seen amongst the control subjects (n=18), but were not exclusively associated with BP reduction, since improvements in LVM index were observed in both responders and non-responders (Brandt, Mahfoud et al. 2012). Mahfoud et al. also reported a reduction in LV mass (46 ± 14 $g/m^{1.7}$ vs. 43 ± 13 $g/m^{1.7}$, p=0.001), and improvements in LVEF (43% vs. 50%, p=0.001) and LV circumferential strain (reported as a surrogate of diastolic function; 215 vs. 218; p= 0.001) as assessed by CMR, in patients treated with RDN (n=55), not seen in controls (n=17) (Mahfoud, Urban et al. 2014). LVM index reduced in both RDN BP responders and non-responders in this MRI based study (Mahfoud, Urban et al. 2014). Delacroix et al. reported improvements in myocardial perfusion and ejection fraction following RDN (Delacroix, Chokka et al. 2018). Improvements in atrial dilatation and atrial electrophysiology have also been reported following RDN (McLellan, Schlaich et al. 2015, Schirmer, Sayed et al. 2015), effects which are not necessarily dependent on BP reduction (Schirmer, Sayed et al. 2015).

T1 mapping techniques have been used to better define the nature of the reduction in LVM index reported in earlier studies, demonstrating a reduction in LV interstitial fibrosis (Doltra, Messroghli et al. 2014, McLellan, Schlaich et al. 2015). In a study of 23 patients undergoing RDN, there was a significant reduction in LVM index (42 ± 10 versus 38 ± 7 $g/m^{1.7}$, p=0.001) and absolute interstitial volume index (10 ± 2 versus 9 ± 2 $mL/m^{1.7}$, p=0.031, with no change in extracellular volume fraction (26 ± 4% versus 26 ± 5%, p=0.61). (Doltra, Messroghli et al. 2014). As with our cohort, Delacroix et al. report a reduction in extracellular volume fraction following RDN (46 ± 4% versus 41 ± 8%, p=0.002) (Delacroix, Chokka et al. 2018). These data support our findings that the reduction in LV mass seen following RDN is not solely due to a reduction in cardiac myocyte hypertrophy, but also due to a reduction in interstitial myocardial fibrosis (Doltra, Messroghli et al. 2014). Once again in this latter study, both BP responders and non-responders had a significant reduction in LVM index as seen in our cohort.

Meta-regression analysis of 12 studies using echocardiography or CMR to assess cardiac structure and function following RDN, supported improvements in LVM index and left atrial volume after the intervention, but failed to demonstrate a significant relationship between RDN-induced LVM index reduction and BP lowering at 6 months (Lu, Wang et al. 2016). The mechanism for the reduction in LVM reported after renal denervation remains to be elucidated, the fact that LVM has been seen to improvement amongst patients who have failed to respond to RDN with a BP reduction, as a reproducible finding across multiple studies would suggest that RDN has a beneficial effect on cardiac remodelling beyond a pure response to BP reduction and decreased afterload. There may be independent effects relating to a reduction in cardiac sympathetic nerve activity. Potential mechanisms, and the relationship between muscle sympathetic nerve activity and changes in LVM index are explored in Section 5.3.3.1.2.

There was no change in aortic compliance or distensibility following RDN amongst the 15 participants with available data, even once analysed by RDN BP-response subgroup (see Table 5-8). There was also no correlation between the change in office SBP at 6-months post-RDN and the change in either aortic compliance or distensibility following the procedure, although the trend in these data may suggest that a reduction in SBP following RDN is associated with a reduced vascular stiffness.

Evaluation of the effect of RDN on vascular stiffness has generated conflicting results. Several studies have reported a reduction in PWV following RDN (Brandt, Reda et al. 2012, Mortensen, Franzen et al. 2012, Baroni, Nava et al. 2015, Palionis, Berukstis et al. 2016, Delacroix, Chokka et al. 2018, Ott, Franzen et al. 2018), including the improvement in PWV seen a cohort of 110 patients reported by Brandt et al. (Brandt, Reda et al. 2012). Hering et al. also reported an improvement in augmentation index (increased with increased vascular stiffness), independent of changes in BP or MSNA, following RDN (Hering, Lambert et al. 2013). In the sham-controlled ReSET study, the RDN participants (n=26) had significant reductions in office BP and pulse wave velocity (PWV; a measure of vascular stiffness), not seen in the control group (n=27), however, overall, there was no significant difference between the sham and RDN outcomes for these parameters (Peters, Mathiassen et al. 2017). In the DENERVHTA study, patients were randomised to treatment with spironolactone versus RDN; there was no change in carotid-femoral PWV amongst those patients treated with RDN (Oliveras, Armario et al. 2018). Furthermore, Verloop et al. actually reported an increase in PWV following RDN (n=57) (Verloop, Vink et al. 2015), and in a study investigating the effect of RDN on markers of micro- and macro-vascular function in patients with heart failure with preserved ejection fraction, Patel et al. reported no change in aortic distensibility or PWV following RDN (Patel, Hayward et al. 2017).

One issue is the variation in the method used to assess vascular stiffness, be it carotid-femoral or carotid-radial PWV (Brandt, Reda et al. 2012, Mortensen, Franzen et al. 2012, Baroni, Nava et al. 2015), measures of augmentation index from applanation tonometry (Mortensen, Franzen et al. 2012, Hering, Lambert et al. 2013), or measurement of aortic distensibility using cross-sectional imaging (Patel, Hayward et al. 2017). Patient selection must also be considered, since data would suggest that patients with isolated systolic hypertension, likely secondary to increased vascular stiffness are less likely to respond to RDN (Ewen, Ukena et al. 2015, Fengler, Rommel et al. 2017), and it may be that patients with longstanding hypertension, and irreversible stiffening of the vasculature are unlikely to respond to the procedure; variation in the effect of RDN on vascular stiffness may reflect variation in the populations studied (Fengler, Rommel et al. 2017).

Most recently, in a multicentre study of 65 patients by Stoiber et al., there was a significant, 33%, improvement in aortic distensibility 1.52 ± 0.82 to $2.02 \pm 0.93 \times 10^{-3}$ $mmHg^{-1}$ ($p < 0.001$) (Stoiber, Mahfoud et al. 2018). In this study, the increase in aortic distensibility was more pronounced in younger patients ($p = 0.005$) and responders to RDN ($p = 0.002$), although aortic distensibility did improve in all age groups following RDN. Interestingly, the improvement in aortic distensibility was not related to changes in BP, suggesting that RDN may have direct effects on the central vasculature, independent of any antihypertensive effect.

5.2.4.6 Limitations

There are some major limitations and assumptions impacting the interpretation of outcome data from this study: there was incomplete ABPM data which would have provided a more robust BP end-point, there were multiple medication changes over the course of the study which may have confounded the data, and medication adherence was not formally confirmed. Hence, we cannot exclude the possibility that the beneficial clinical outcomes seen in this study are due to improved medication adherence and a Hawthorne effect.

5.2.5 Conclusions

The safety of renal denervation was supported by this pilot study of 18 patients. The reduction in office SBP at 6 months after denervation failed to achieve significance, but did attain significance at 12 months after the intervention. Importantly, RDN also positively impacted target organ damage, reducing LV mass, and preventing, in those with a reduction in BP following RDN, the progressive decline in renal function seen in non-responders.

It is interesting to note that a significant reduction in office SBP was not attained until 12 months after the procedure. This observation echoes the sustained and progressive reduction in SBP reported in earlier studies including Symplicity HTN-1 (Krum, Schlaich et al. 2014). Looking over this timescale, RDN is likely to have an affect beyond an acute reduction in sympathetic nerve activity and vascular tone. The mechanism for this delayed antihypertensive effect of RDN is not clear but may represent vascular remodelling and changes in vascular stiffness, gradual resetting of the baroreflex or the sensitivity of sympathovascular transduction, or slow shifts in the balance of the renin-angiotensin-aldosterone system (Krum, Schlaich et al. 2014). In this cohort, in contrast to data from a recent multicentre study (Stoiber, Mahfoud et al. 2018), there was no change in aortic distensibility at 6 months post-RDN, and it is possible that assessment of vascular stiffness at a greater interval from the procedure would have given more time for remodelling to occur, although this is clearly speculation.

Despite the limitations impacting this study, these pilot data do support the use of RDN for the treatment of resistant hypertension on the basis of clinical outcomes, but further investigation is required in the context of large-scale studies, with a particular focus on the potential to improve target organ damage, and thereby improve cardiovascular morbidity and mortality. These data also start to give insight into factors which may help to predict a clinical response to RDN, including factors such as established vascular stiffness, which will be explored further in Section 5.6 of this manuscript.

5.3 Impact of renal denervation on sympathetic nerve activity

5.3.1 Introduction

Sympathetic nerve activity (SNA) controls vasomotor tone in peripheral blood vessels and has been shown to be elevated in patients with hypertension (Yamada, Miyajima et al. 1989). It has been hypothesised that renal denervation (RDN) can reduce systemic blood pressure through disruption of efferent sympathetic input to the kidney, thereby reducing renal vasoconstriction, improving renal blood flow and reducing renin release and Na^+ and water reabsorption (Sobotka, Mahfoud et al. 2011). Alternatively, or as a parallel mechanism, removal of endogenous afferent activity in the renal nerves could reduce sympathetic tone generally, and thereby blood pressure. This is based on the idea that afferent activity provides a major drive to sympathetic tone generation via reflex pathways (Koeners, Lewis et al. 2016, Patinha, Pijacka et al. 2017). In conditions of hypertension, renal afferents may become activated in response to local ischaemia (Nijima 1971, Winternitz, Katholi et al. 1980, Johns, Kopp et al. 2011, Koeners, Lewis et al. 2016). In this study we aimed to evaluate the effect of RDN on SNA, and to assess whether elevated SNA is a predictor for a blood pressure lowering response to RDN, by quantifying multi-unit muscle sympathetic nerve activity (MSNA) using a technique called microneurography. The background to this technique, which was initially established by Hagbarth and Vallbo in the mid-1960s (Hagbarth and Vallbo 1968, Vallbo, Hagbarth et al. 2004), is discussed in Section 2.1.2.1.

Microneurography is a specialist technique which can give temporally dynamic and reproducible quantification of MSNA (Hart, Joyner et al. 2010, Hart, Head et al. 2017), however, the technique is invasive, can be time consuming, and requires an experienced operator. Consequently, efforts have been made to find alternative, non-invasive markers of SNA. One such measure is heart rate variability (HRV).

The sinus node and RR interval are under continuous modulation through opposing parasympathetic vagal activity and cardiac afferent sympathetic activity. This efferent sympathetic and vagal nerve activity can be modulated, in part, by central (vasomotor and respiratory centers) and peripheral (oscillation in arterial pressure and respiratory movements) oscillators (1996). These oscillators generate rhythmic fluctuations in nerve activity that manifest as short- and long-term oscillations in the RR interval, analysis of which may facilitate indirect inferences about cardiac sympathetic and vagal efferent activity (1996). HRV can be analysed in both time and frequency domains. Spectral analysis of the HRV frequency domain distinguishes three main components from short-term recordings; very low frequency (VLF), low frequency (LF), and high frequency (HF) components. Vagal activity is the major contributor to the HF component, and at rest the sinus node is predominantly under the control of vagal tone. The LF component is reported to be a quantitative marker of sympathetic modulation, and there is a predominance of LF oscillations during sympathetic activation (Pagani, Montano et al. 1997). The clinical implications for measures of HRV rose to the fore after the publication of data showing that reduced HRV was associated with increased cardiovascular mortality following acute myocardial infarction (Kleiger, Miller et al. 1987). Data from the Framingham Heart Study have shown reduced HRV in individuals with hypertension, and that impaired HRV predicted the development of hypertension in normotensive men (Singh, Larson et al. 1998). However, the strength of HRV as a

method to assess SNA remains controversial, including data which fail to show a consistent correlation between MSNA and LF power (Saul, Rea et al. 1990, DeBeck, Petersen et al. 2010). For example, Saul et al. investigated the effect of graded infusions of nitroprusside and phenylephrine on MSNA and the power spectral measures of HRV (Saul, Rea et al. 1990). At baseline, there was no correlation between any of the HRV spectral measures and MSNA. During infusion of nitroprusside there were increases in both MSNA and the LF fraction of the power spectral analysis, conversely, during the phenylephrine infusion there was a reduction in MSNA, however, no HRV component correlated with this change in MSNA. The investigators conclude that LF fluctuations in HR results from changing levels in both sympathetic and parasympathetic drive (Saul, Rea et al. 1990). Therefore, it may be that the LF spectral component reflects both sympathetic and vagal activity, rather than acting as a pure marker of SNA, and that the LF/HF ratio gives a better indication of overall sympathovagal balance (Rimoldi, Pierini et al. 1990, Montano, Ruscone et al. 1994, 1996). The physiological interpretation of VLF and ultra-low frequency components of the spectral analysis still requires further investigation, furthermore, these components are difficult to interpret from short-term recording such as those performed in this study (1996). Importantly, HRV measures changes in autonomic activity, or sympathovagal balance, rather than the absolute level of sympathetic or vagal tone and could be blunted at extremely high (saturating) levels of SNA. The question is whether reductions in SNA would produce measurable changes in sympathovagal balance, and thus LF or LF/HF ratio, in patients with extremely high (saturating) levels of MSNA as seen in some individuals with hypertension? Furthermore, it is not clear whether HRV reflects merely a marker of cardiovascular disease severity, or whether it measures pathological changes in autonomic function (Eckberg 2000).

In this study we aimed to evaluate the effect of RDN on sympathetic nerve activity, as assessed by either MSNA or HRV, and whether changes in these parameters correlated with the BP response to RDN. We also assessed whether elevated SNA was a predictor of response to RDN, and these data are presented in Section 5.6. In addition to these previously stated aims, we will consider whether HRV, an indicator of cardiac sympathetic nerve activity, correlates with muscle SNA in this context, or whether these organ specific measures of SNA are differentially regulated.

5.3.2 Methods

5.3.2.1 Microneurography

The methods for performing microneurography and the analysis of MSNA data are described in Section 4.3.6.

5.3.2.2 Heart rate variability

Heart rate variability (HRV) was analysed from a 5 minute 3-lead ECG recording using commercially available software (LabChart, AD Instruments, Dunedin, New Zealand). The

automated marking of each ECG recording was visually reviewed to ensure the accurate marking of all R waves and identification of any ectopic beats. In a continuous ECG record, the normal-to-normal (NN) intervals (all intervals between adjacent QRS complexes resulting from sinus node depolarisations) were determined. Time domain variables were calculated, including the standard deviation of the NN intervals (SDNN), which equates to the square root of variance. SDNN reflects all the cyclic components responsible for variability in the period of recording, and is an estimate of overall HRV (variance is mathematically equal to the total power from spectral analysis), however, it should be noted that with shorter recording lengths such as the 5 min recording used in this study, SDNN will represent shorter cycle lengths (Electrophysiology 1996). Other measures derived from the RR interval differences included RMSSD, the square root of the mean squared differences of successive NN intervals, NN50, the number of interval differences of successive NN intervals greater than 50 ms, and pNN50, the NN50 count divided by the total number of all NN intervals. All of these measures estimate short-term, and therefore high-frequency, variations in heart rate and are highly correlated (Electrophysiology 1996).

A range of parameters can be derived from the power spectral analysis, by Fast Fourier Transform, of a 5 minute ECG recording; these components are summarised in Table 5-9 (1996). The measurement of VLF, LF, and HF power components is made in absolute values of power (milliseconds squared). LF and HF can also be quantified in normalised units (the proportion of the respective power value relative to the total power minus the VLF component), which reduced the effect of changes in total power on the values of the LF and HF components. The LF/HF ratio may also better reflect the relative balance between cardiac sympathetic and vagal activity.

Variable	Units	Description	Frequency range
Total power	ms^2	The variance of NN intervals over the temporal segment	approximately ≤0·4 Hz
VLF	ms^2	Power in very low frequency range	≤0·04 Hz
LF	ms^2	Power in low frequency range	0·04–0·15 Hz
nLF	n.u.	LF power in normalised units	
HF	ms^2	Power in high frequency range	0·15–0·4 Hz
nHF	n.u.	HF power in normalised units	
LF/HF		Ratio LF/HF	

Table 5-9. Selected frequency domain measures of heart rate variability for the analysis of short-term (5 min) recordings.
Adapted from ESC guidelines for Heart rate variability: Standards of measurement, physiological interpretation, and clinical use (Electrophysiology 1996).

5.3.2.3 Estimated total peripheral resistance

The method for the quantification of total peripheral resistance (TPR) is given in Section 5.2.3.4.

5.3.2.4 Target organ damage

MSNA and HRV data have been correlated against measures of target organ damage as assessed by cardiac magnetic resonance imaging. The methods for the CMR imaging and analysis are described in Section 4.3.4.

5.3.3 Results

5.3.3.1 MSNA

MSNA was successfully recorded in 14/18 participants at baseline (78% of subjects; unable to record an adequate quality neurogram in 3/18 patients, microneurography not recorded in 1 participant due to time constraints). Baseline MSNA incidence was 60 ± 6 bursts/100 heart beats, and baseline MSNA frequency was 38 ± 3 bursts/min. There was an *inverse* correlation between baseline office SBP and baseline MSNA incidence (see Figure 5-14). Baseline heart rate was inversely correlated with baseline MSNA incidence (R=-0.63, p=0.02, see Figure 5-14), but did not correlate with baseline MSNA frequency (R=-0.14, p=0.63).

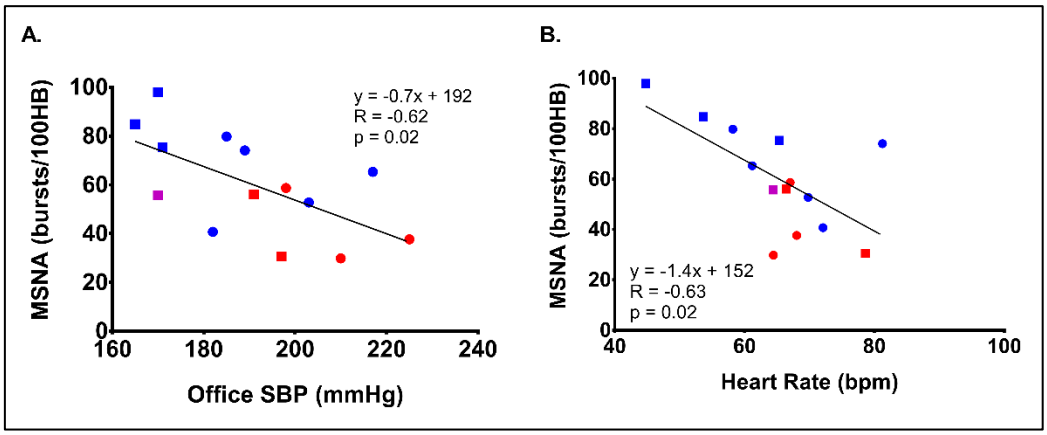


Figure 5-14. Negative correlations between A. office systolic blood pressure (SBP) and B. resting heart rate, and muscle sympathetic nerve activity (MSNA) incidence at baseline prior to renal denervation (RDN).
Male participants shown in blue, premenopausal women in red and postmenopausal women in purple. Data from RDN BP-responders (as defined by office SBP reduction ≥10 mmHg at 6 months post-RDN) are shown as dots and data from RDN non-responders are shown as squares.

MSNA data were available at baseline and 6 months post-RDN in 11 participants. Amongst this group of patients there was no significant change in any of the MSNA parameters assessed at this primary endpoint (see Table 5-10).

Parameter	Time post RDN (months)	P

	0	6	
MSNA incidence (bursts/100 HB)	61 ± 7	66 ± 5	0.47
MSNA frequency (bursts/min)	38 ± 4	41 ± 3	0.48
Total MSNA area/100 HB (%/s)	3351 ± 411	3677 ± 355	0.55
Total MSNA area/min (%/s)	2075 ± 198	2283 ± 198	0.49

Table 5-10. Muscle sympathetic nerve activity (MSNA) at baseline and 6 months after renal denervation (RDN).
100HB; 100 heart beats. P values are for paired Student's t-test for the 11 participants with MSNA data at baseline and 6 months post-RDN.

There was no change in MSNA following RDN by any of the SNA parameters measured over the course of the study as assessed by repeated-measures ANOVA with data carried forward (n=14, see Table 5-11 and Figure 5-15). There was no correlation between the change in office SBP at 6 months post-RDN and the change in either MSNA incidence or MSNA frequency at 6 months post RDN (R=-0.11, p=0.72 and R=-0.04, p=0.89, respectively). Likewise, there was no correlation between the change in resting heart rate and the change in either MSNA incidence or frequency, at 6 months post-RDN (R=-0.49, p=0.13 and R=-0.12, p=0.72, respectively). Examples of MSNA recordings from an individual patient before, and 12 months after RDN, are shown in Figure 5-16.

There was also no change in MSNA burst incidence or burst frequency when data were analysed by response group (analysis by repeated-measures ANOVA with data carried forward, see Table 5-12), and there was no difference in MSNA burst incidence or MSNA burst frequency between responders and non-responders at any study time-point (all p>0.05). Plots for the office SBP and MSNA incidence for each participant (with MSNA data available) are shown in Figure 5-17; in some individuals the change in SBP over the course of the study does seem to have a concordant temporal relationship with the change in MSNA, whilst in others there is no clear relationship between these physiological variables.

Parameter	**Time post RDN (months)**					**P**
	0	1	3	6	12	
MSNA incidence (bursts/100 HB)	60 ± 6	61 ± 6	66 ± 6	66 ± 5	69 ± 5	0.23
MSNA frequency (bursts/min)	38 ± 3	38 ± 3	40 ± 4	41 ± 3	42 ± 3	0.48
Total MSNA area/100 HB (%/s)	3261 ± 355	3581 ± 369	3871 ± 311	3724 ± 323	3870 ± 409	0.42
Total MSNA area/min (%/s)	2050 ± 179	2211 ± 218	2231 ± 198	2319 ± 189	2379 ± 258	0.57

Table 5-11. Measures of muscle sympathetic nerve activity (MSNA) at baseline, and over 12-month follow-up following renal denervation (RDN).
100HB; 100 heart beats. P values are for repeated measures 1-way ANOVA for the 14 participants with MSNA data at baseline; for missing values the result from previous time-point was carried forward. There were no significant differences on between group analysis by Bonferroni's multiple comparison test.

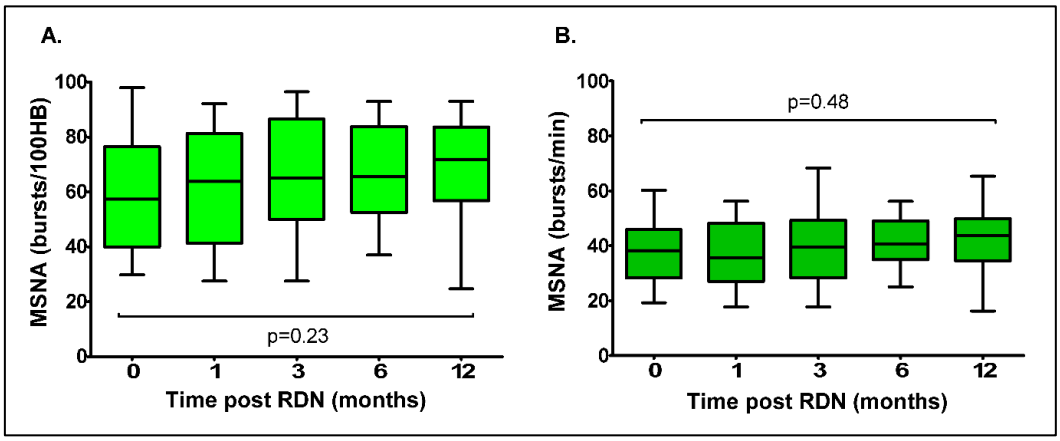


Figure 5-15. Muscle sympathetic nerve activity (MSNA) before (0 months) and following renal denervation (RDN).

100HB; 100 heart beats. P values are for repeated measures 1-way ANOVA for the 14 participants with MSNA data at baseline; for missing values the result from previous time-point was carried forward. There were no significant differences on between group analysis by Bonferroni's multiple comparison test.

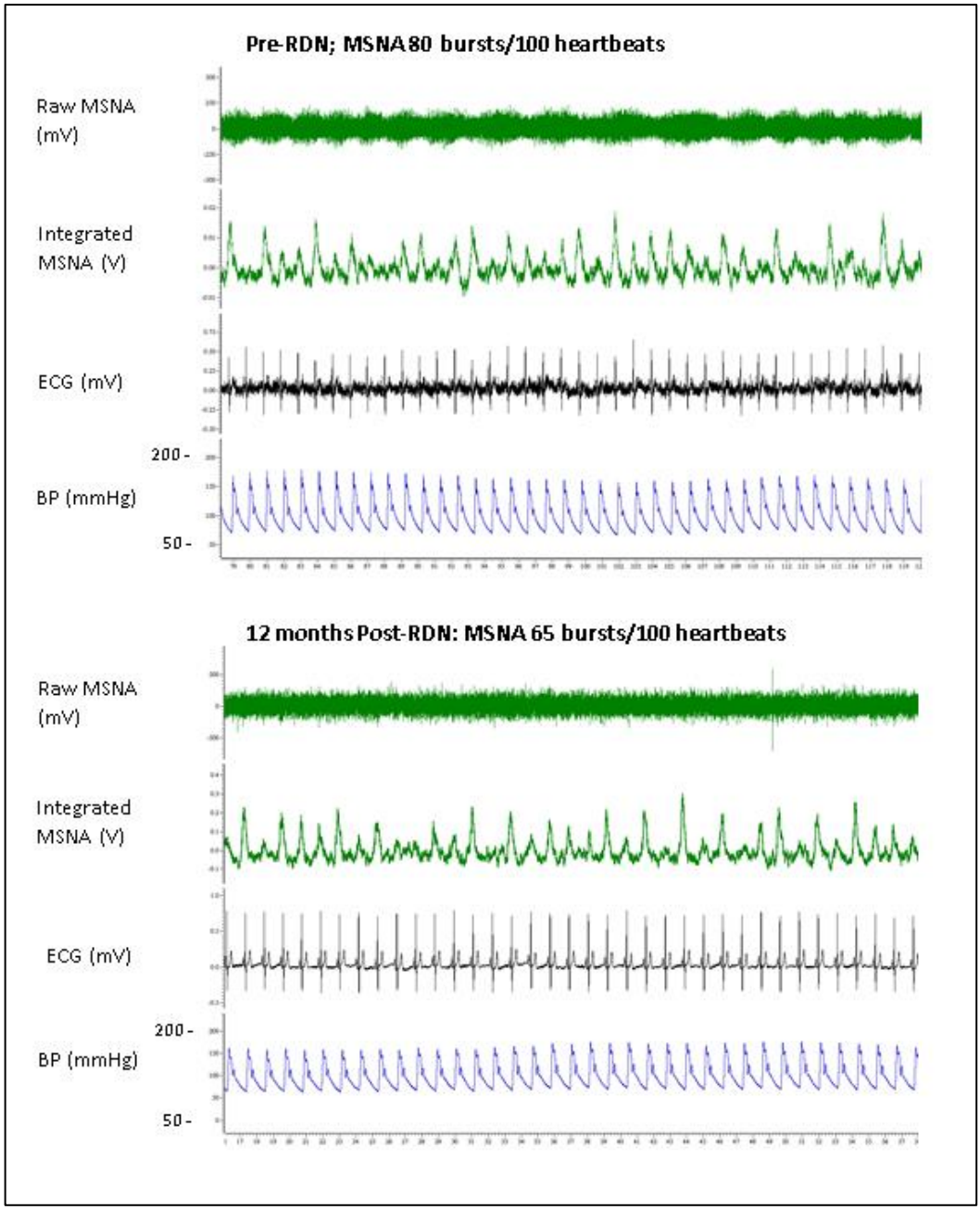
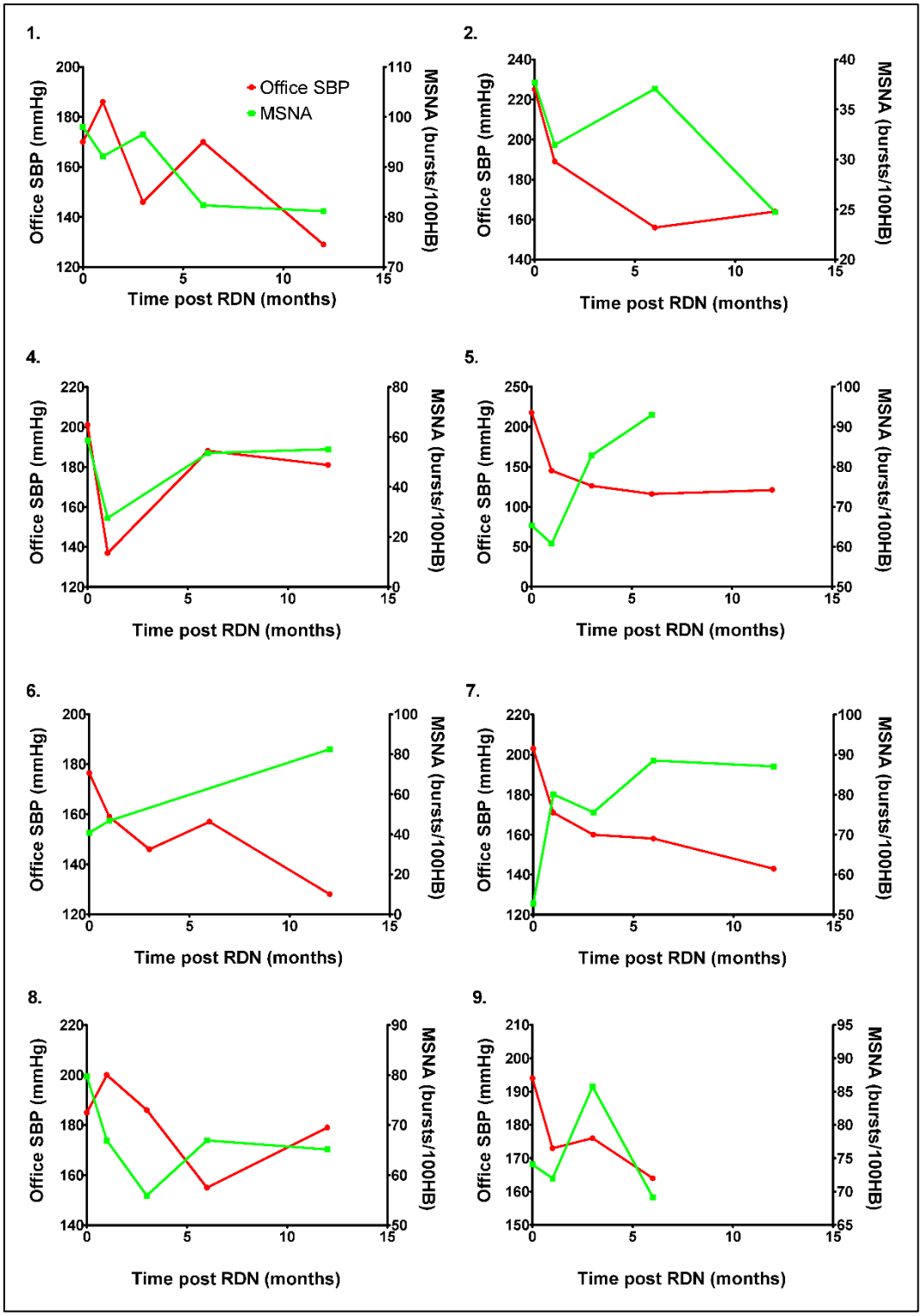


Figure 5-16. Muscle sympathetic nerve activity recorded before (top figure), and 12 months after (bottom figure), renal denervation (RDN) in an individual patient.
Data shown are for participant no. 8; this patient responded to RDN with a change in office systolic blood pressure (oSBP) of -30 mmHg at 6 months post-RDN.


1.
Office SBP
MSNA
Office SBP (mmHg)
MSNA (bursts/100HB)
Time post RDN (months)
2.
Office SBP (mmHg)
MSNA (bursts/100HB)
Time post RDN (months)
4.
Office SBP (mmHg)
MSNA (bursts/100HB)
Time post RDN (months)
5.
Office SBP (mmHg)
MSNA (bursts/100HB)
Time post RDN (months)
6.
Office SBP (mmHg)
MSNA (bursts/100HB)
Time post RDN (months)
7.
Office SBP (mmHg)
MSNA (bursts/100HB)
Time post RDN (months)
8.
Office SBP (mmHg)
MSNA (bursts/100HB)
Time post RDN (months)
9.
Office SBP (mmHg)
MSNA (bursts/100HB)
Time post RDN (months)

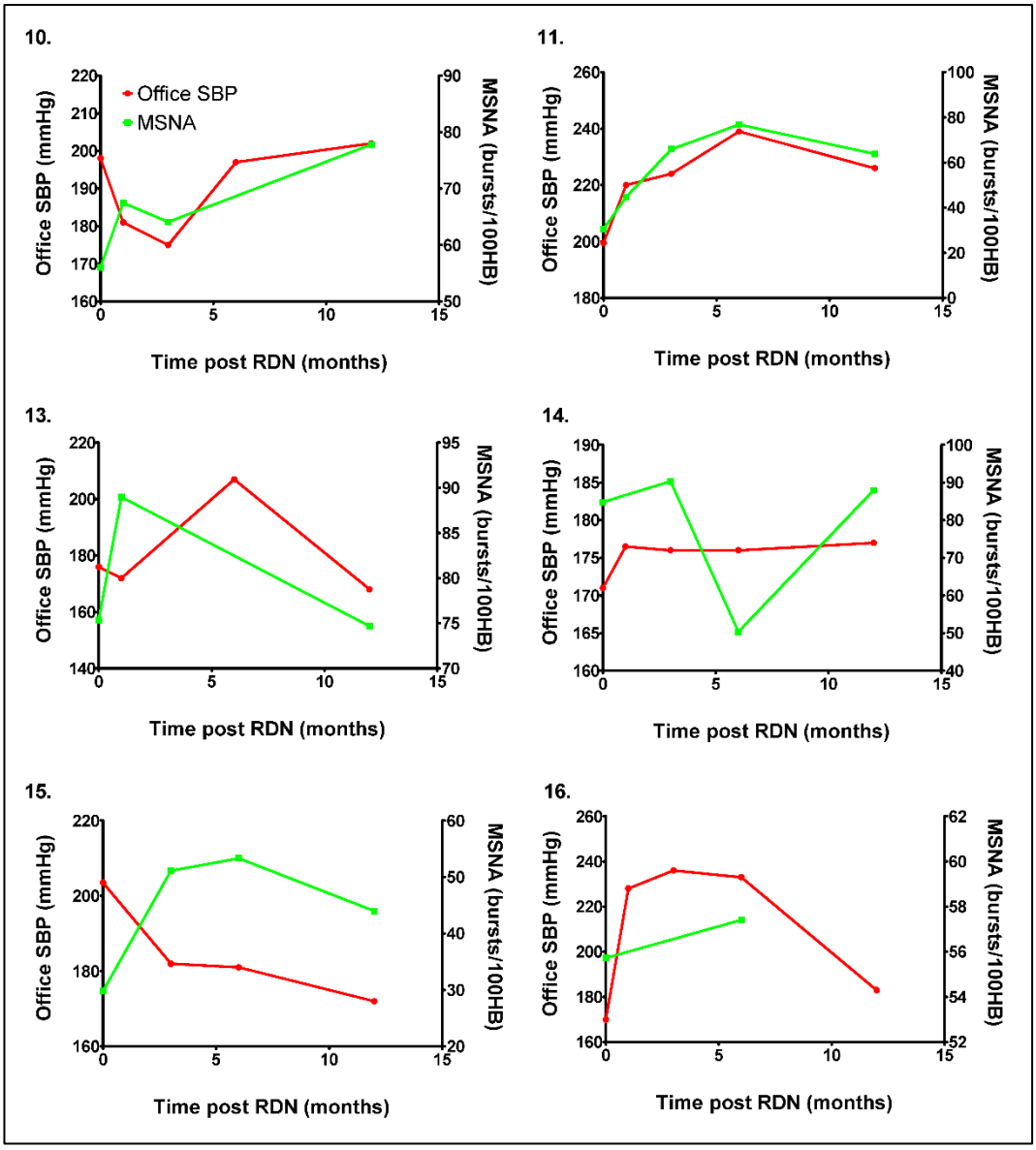


Figure 5-17. Individual data for office systolic blood pressure (SBP) and muscle sympathetic nerve activity (MSNA) over the 12 months following renal denervation (RDN).

Office SBP is shown in red and MSNA is shown in green. The patient numbers for each figure are consistent with those used throughout the manuscript. Patient 9 withdrew from the study prior to 12-month follow-up. Bursts/100HB; bursts/100 heart beats.

Parameter	Time post RDN (months)					P
	0	1	3	6	12	

MSNA incidence (bursts/100 HB)						
Responders	55 ± 6	52 ± 7	57 ± 8	64 ± 7	65 ± 8	0.17
Non-responders	67 ± 10	72 ± 8	77 ± 7	70 ± 6	74 ± 5	0.65
MSNA frequency (bursts/min)						
Responders	37 ± 5	33 ± 5	37 ± 6	41 ± 4	40 ± 5	0.47
Non-responders	39 ± 4	43 ± 4	45 ± 3	42 ± 4	45 ± 2	0.35

Table 5-12. Muscle sympathetic nerve activity (MSNA) following renal denervation (RDN) by blood pressure response group.
100HB; 100 heart beats. P values are for repeated measures 1-way ANOVA for the 14 participants with MSNA data at baseline (responders, n=8; non-responders, n=6); for missing values the result from previous time-point was carried forward. There were no significant differences in between group analyses.

5.3.3.1.1 Relationship between MSNA and total peripheral resistance

There was no correlation between MSNA incidence and estimated TPR at baseline (n=14, R=0.08, p=0.77, see Figure 5-18). There was no correlation between the change in MSNA incidence and the change in estimated TPR at 6 months post-RDN (n=11, R=-0.23, p=0.49, see Figure 5-18).

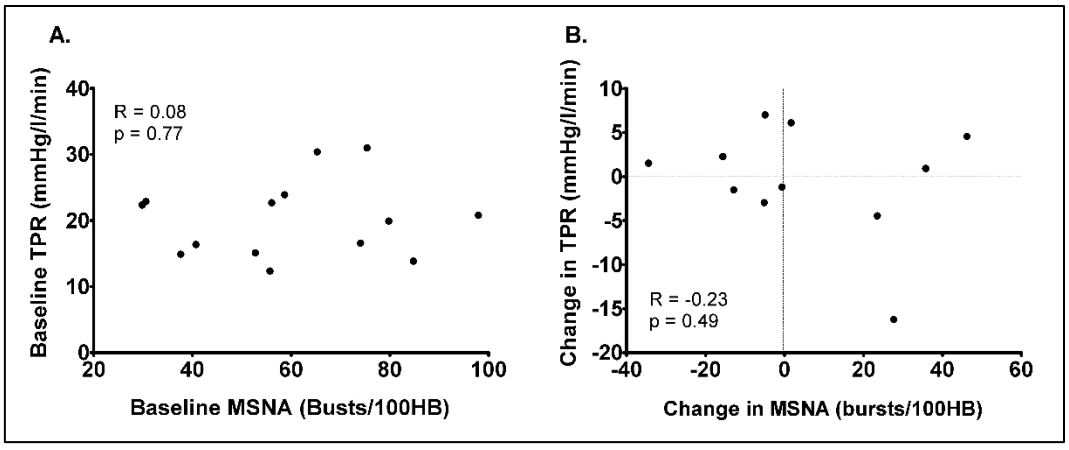


Figure 5-18. Relationship between muscle sympathetic nerve activity (MSNA) and total peripheral resistance (TPR) at baseline (A.), and the relationship between changes in these parameters 6 months post-RDN.
Data are shown for the 14 participants with available baseline data, and the 11 patients with available 6-month follow-up data. MSNA is reported in bursts/100 heart beats (HB).

5.3.3.1.2 MSNA and target organ damage

At baseline (n=14), the correlation between MSNA and indexed left ventricular mass (LVM) did not achieve significance (R=0.44, p=0.12), however there was a significant correlation between both peak radial strain and peak circumferential strain and MSNA (R=-0.60, p=0.02 and R=0.58, p=0.03, respectively) and a trend towards a correlation between peak longitudinal strain and MSNA (R=0.48, p=0.08, see Figure 5-19). To summarise, at baseline, increased MSNA correlated with impaired/reduced peak radial

strain (thickening) and reduced peak circumferential (shortening), and participants with higher MSNA may have a possible trend towards increased LVM index. Peak systolic and diastolic radial, circumferential and longitudinal strain rates showed a similar pattern of correlation with MSNA incidence at baseline: systolic, R=-0.66, p=0.01; R=0.63, p=0.02; R=0.49, p=0.07, diastolic, R=0.58, p=0.03; R=-0.60, p=0.02; R=-0.73, p=0.003, respectively (note, only trend towards a correlation between baseline MSNA and baseline peak systolic longitudinal strain rate). These data would suggest that prior to RDN, raised MSNA was associated with impaired LV function.

MSNA was measured following renal denervation; 11 of the 14 participants with baseline MSNA data had MSNA data of sufficient quality recorded 6 months post-RDN. There was no correlation between the change in MSNA incidence and the change in LVM index at 6 months post-RDN (R=0.08, p=0.81, Figure 5-20). At 6 months, there was a significant correlation between the change in stroke volume index and the change in MSNA incidence (R=0.66, p=0.03, Figure 5-20); those patients with a decrease in MSNA post-RDN had a decrease in stroke volume post-RDN. When comparing the changes in MSNA and strain parameters following RDN, the only correlation which approached significance was that between the change in MSNA incidence and the change in peak systolic longitudinal strain rate (R=0.59, p=0.05, Figure 5-20), with data indicating that a decrease in MSNA following RDN may be associated with increased (more negative) peak longitudinal strain rate. In summary, whilst LVM did decrease post-RDN (see Table 5-6), this reduction was not associated with a change in MSNA, there was also no definite correlation between any potential improvement in LV function following RDN and a change in MSNA, although a decrease in stroke volume following RDN was associated with a reduction in MSNA.

There was no correlation between baseline MSNA and either baseline aortic compliance or distensibility (n=11; R=0.22, p=0.53 and R=0.10, p=0.78, respectively). Likewise, there was no correlation between the change in MSNA and either the change in aortic compliance or distensibility at 6 months post-RDN (n=8; R=0.12, p=0.77 and R=0.17, p=0.70, respectively.

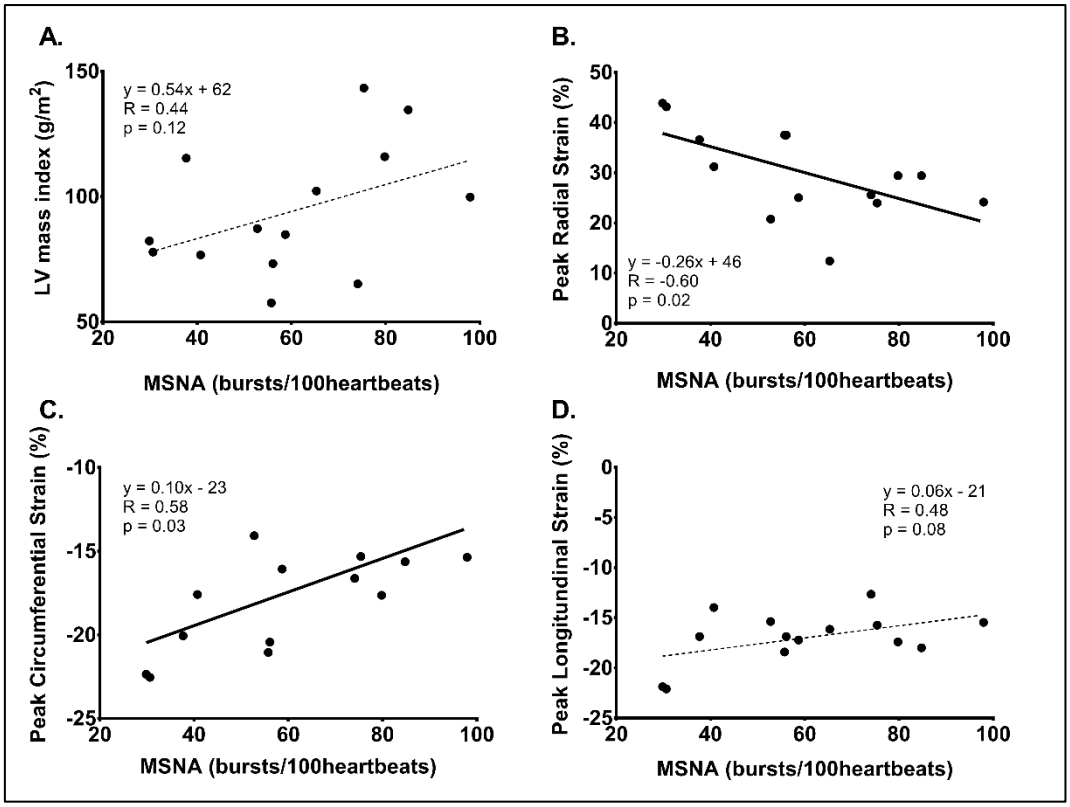


Figure 5-19. Correlations between baseline muscle sympathetic nerve activity (MSNA) and baseline measures of cardiac structure and function.

Data presented for the 14 participants with baseline MSNA, for baseline MSNA incidence versus A. Left ventricular mass index, B. Peak radial strain, C. Peak circumferential strain and D. Peak longitudinal strain. P<0.05 was taken to indicate significance.

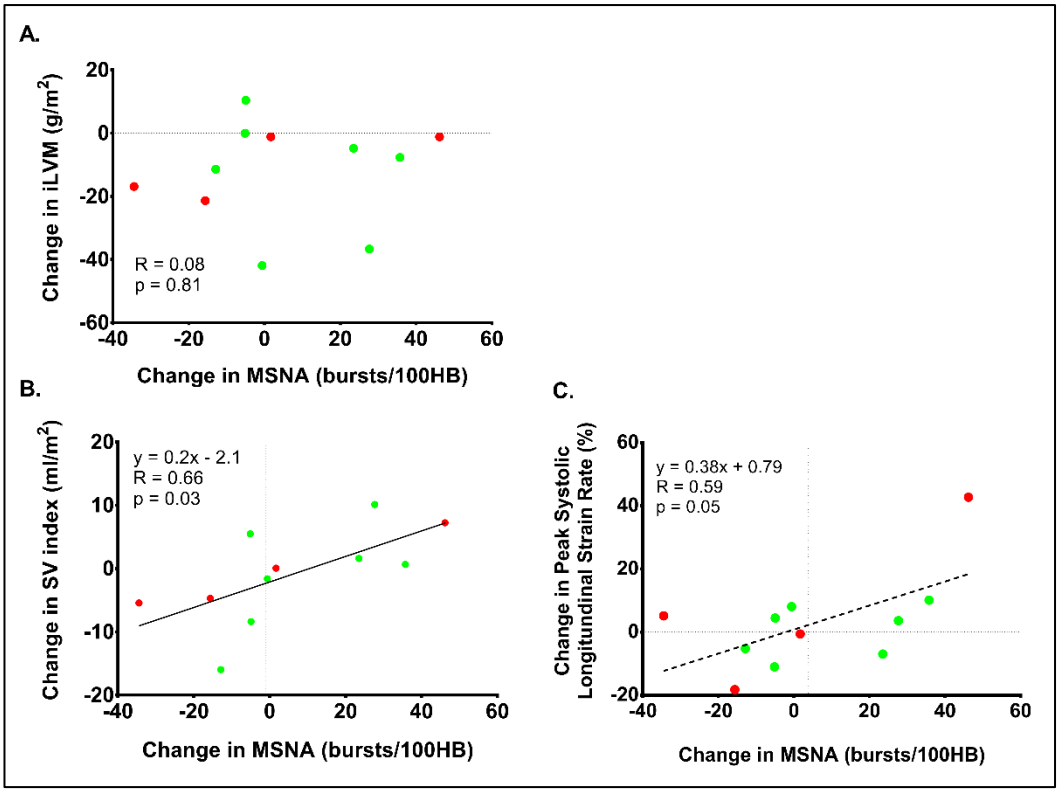


Figure 5-20. Relationships between the change in muscle sympathetic nerve activity (MSNA) and change in A. indexed left ventricular mass (iLVM), B. indexed stroke volume (SV), and C. peak systolic longitudinal strain rate, 6 months after renal denervation.
Results presented for the 11 patients with follow-up MSNA data at 6 months post-RDN. Data for RDN BP-responders (reduction in office SBP ≥10 mmHg at 6 months post-RDN) shown in green, data for RDN BP non-responders shown in RDN.

5.3.3.2 Heart rate variability

Baseline HRV data were recorded in 17/18 participants (one participant did not have an adequate quality 5 min ECG recording). There were no significant correlations between any of the baseline HRV parameters and either baseline office SBP data (n=17), baseline resting heart rate (HR; n=17), or baseline MSNA data (n=14, all p>0.05); this may support the concept that sympathetic outflow is differentially regulated between organs, with differential input to the peripheral vasculature and myocardium (Esler, Jennings et al. 1984).

HRV was quantified at 16 participants at both baseline and the primary outcome timepoint of 6 months post-RDN. There were no significant changes in any of the HRV parameters between baseline and 6 months as assessed by paired Student's t-test (see Table 5-13). The HRV data as assessed by repeated-measures ANOVA with data carried forward, are summarised in Table 5-14; there were no significant changes in any of the measures of HRV across the study follow-up visits by this statistical method.

At 6 months post-RDN, there were no correlations between the change in office SBP and the changes in any of the HRV parameters (n=16, all p>0.05). There were no correlations

between changes in any of the HRV spectral frequency band data and the change in MSNA incidence at 6 months after RDN (n=11, all p>0.05), however, there were inverse correlations between the changes in NN50 and pNN50 and the change in MSNA incidence at this time point (n=11; R=-0.71, p=0.02 and R=-0.68, p=0.02, respectively, see Figure 5-21). Given that NN50 and pNN50 results are felt to represent high frequency spectral data, this association would suggest that those individuals with an increase in cardiac vagal tone following RDN also had a decrease in muscle SNA, and thus suggests that any sympathoinhibitory effect of RDN may impact multiple organs. As described above, and as can be seen from Figure 5-21, these changes are independent of changes in SBP.

Parameter	Time post RDN (months)		P
	0	6	
SDNN (ms)	43 ± 5	54 ±8	0.15
RMSSD (ms)	34 ±6	57 ±14	0.14
NN50 (n=)	19 ±6	38 ±11	0.15
pNN50 (%)	6 ±2	13 ±4	0.10
Total power (ms^2)	2073 ±435	3573 ±905	0.14
VLF (ms^2)	827 ±226	1087 ±228	0.33
LF (ms^2)	448 ±112	726 ±316	0.37
nLF (n.u.)	45 ±7	43 ±8	0.77
HF (ms^2)	496 ±148	1024 ±337	0.15
nHF (n.u.)	40 ±5	43 ±5	0.64
LF/HF	2.1 ±0.7	1.7 ±0.4	0.61

Table 5-13. Measures of heart rate variability (HRV) at baseline versus 6 months after renal denervation (RDN).

SDNN; standard deviation of differences between successive NN (normal to normal) intervals, RMSSD; square root of the mean squared differences of successive NN intervals, NN50; number of interval differences of successive NN intervals measuring >50 ms, pNN50; NN50 count as a percentage of the total number of all NN intervals, VLF; very low frequency, LF; low frequency, nLF; normalised low frequency, HF; high frequency, nHF; normalised high frequency. P values are for a paired Student's t-test, n=16.

Parameter	Time post RDN (months)					P
	0	1	3	+6	12	
SDNN (ms)	44 ± 4	47 ± 6	58 ± 12	54 ± 7	51 ± 7	0.74
RMSSD (ms)	38 ± 6	45 ± 10	60 ± 23	55 ± 14	53 ± 13	0.96
NN50 (n=)	18 ± 6	27 ± 10	24 ± 8	36 ± 11	25 ± 9	0.65
pNN50 (%)	5 ± 1	9 ± 3	9 ± 3	12 ± 4	9 ± 3	0.73
Total power (ms^2)	2041 ± 410	2817 ± 895	5079 ± 2934	3461 ± 858	3307 ± 1188	0.43
VLF (ms^2)	830 ± 213	903 ± 258	1065 ± 235	1105 ± 215	809 ± 166	0.14
LF (ms^2)	439 ± 105	625 ± 262	669 ± 255	694 ± 298	419 ± 101	0.95
nLF (n.u.)	45 ± 7	47 ± 6	52 ± 6	44 ± 8	45 ± 7	0.79

HF (ms^2)	480 ± 140	986 ± 532	2632 ± 2178	968 ± 321	1583 ± 921	0.87
nHF (n.u.)	39 ± 5	39 ± 4	38 ± 5	42 ± 5	43 ± 5	0.98
LF/HF	2.0 ± 0.6	1.7 ± 0.3	2.5 ± 0.7	1.7 ± 0.4	1.6 ± 0.3	0.92

Table 5-14. Measures of heart rate variability (HRV) at baseline, and over 12-month follow-up following renal denervation (RDN).
SDNN; standard deviation of differences between successive NN (normal to normal) intervals, RMSSD; square root of the mean squared differences of successive NN intervals, NN50; number of interval differences of successive NN intervals measuring >50 ms, pNN50; NN50 count as a percentage of the total number of all NN intervals, VLF; very low frequency, LF; low frequency, nLF; normalised low frequency, HF; high frequency, nHF; normalised high frequency. P values are for a non-parametric, repeated-measures 1-way ANOVA for the 17 participants with HRV data at baseline; for missing values the result from the previous time-point was carried forward. There were no significant differences for between group analyses for all timepoints.

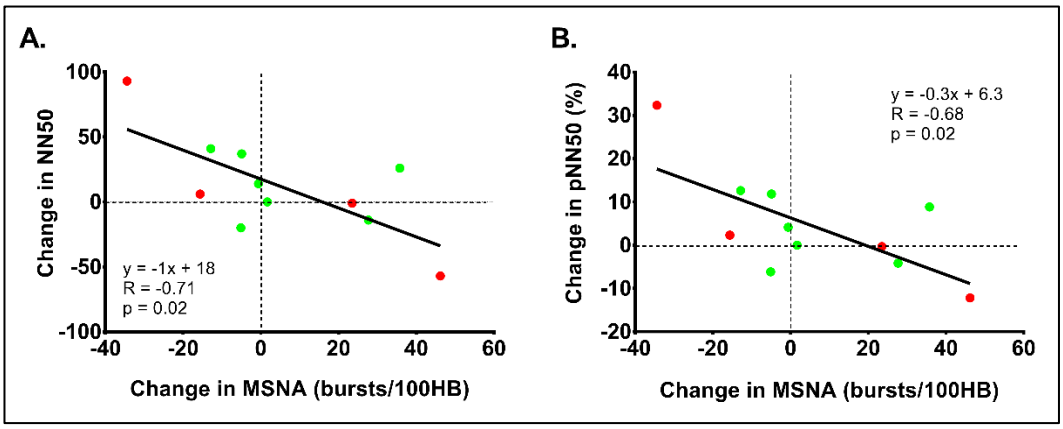


Figure 5-21. Correlations between the change in MSNA incidence and the changes in NN50 and pNN50 at 6 months after renal denervation.
MSNA; muscle sympathetic nerve activity, NN50; number of normal-normal intervals measuring >50 ms, pNN50; proportion of normal-normal intervals measuring >50 ms. Data for RDN BP-responders (reduction in office SBP ≥10 mmHg at 6 months post-RDN) shown in green, data for RDN BP non-responders shown in RDN.

There were no differences in any of the HRV parameters between RDN responders and non-responders at baseline (all p>0.05), however, at 6 months post-RDN, there was a significant difference in mean nHF between responders (n=10) and non-responders (n=6, no 6-month HRV data for one non-responder); nHF was 34 ± 5 n.u. vs 57 ± 7 n.u. respectively (p=0.02). Mean nHF at baseline was 39 ± 5 n.u., which would suggest that, if anything, non-responders had had a relative increase in nHF, and therefore vagal tone, following RDN, however, analysis by 1-way ANOVA, showed no change in nHF over the course of the study amongst either responders (p=0.50) or non-responders (p=0.83), and therefore any inferences from this finding should be interpreted with caution. The difference in the LF/HF ratio between response groups also approached significance (2.2 ± 0.6 vs 0.7 ± 0.3, responders vs non-responders, respectively, p=0.05), when compared to the mean baseline LF/HF result for the whole cohort (2.0 ± 0.6) this could indicate and increase in vagal tone and/or a decrease in cardiac SNA following RDN primarily

amongst the non-responders, but once again, analysis by 1-way ANOVA, showed no change in the LF/HF ratio amongst either responders (p=0.82) or non-responders (p=0.94), and therefore limited conclusions can be drawn.

5.3.3.2.1 Heart rate variability and target organ damage

At baseline (n=17), there were significant correlations between peak longitudinal strain and both nLF and LF/HF HRV parameters (R=0.61, p=0.01 and R=0.62, p=0.007, respectively, see Figure 5-22), indicating that longitudinal cardiac function is impaired in patients with raised HRV spectral markers of increased sympathetic tone and sympathovagal balance. Similarly, there was also a significant correlation between peak systolic longitudinal strain rate and baseline nLF spectral power (R=0.51, p=0.04). Baseline VLF spectral power correlated with baseline peak diastolic radial (R=0.53, p=0.03) and diastolic circumferential (R=-0.54, p=0.03) strain rate, however, given the debate over the factors influencing the VLF spectrum, these data are difficult to interpret. There were no other significant correlations between HRV and CMR measures of myocardial structure and function at baseline.

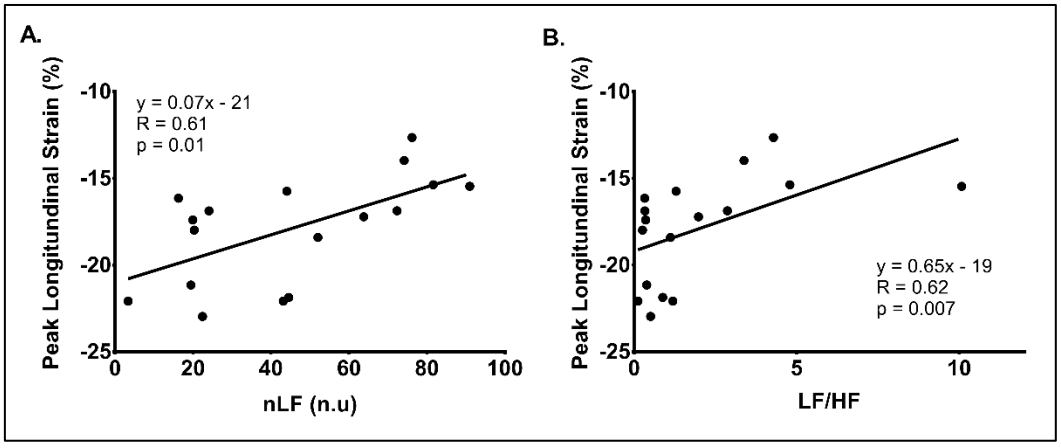


Figure 5-22. Correlations, at baseline, between peak longitudinal strain as assessed by cardiac magnetic resonance imaging, and A. normalised low frequency (nLF) spectral power and B. low frequency to high frequency power ratio (LF/HF).
Data (n=17), indicate that longitudinal cardiac contractility/function is impaired in patients with raised HRV spectral markers of increased sympathetic tone and sympathovagal balance.

The data assessing the relationships between changes in HRV parameters versus changes in cardiac ejection fraction (EF) and volumetric parameters at 6 months post-RDN are summarised in Table 5-15. In summary, changes in markers of vagal tone (time domain parameters and HF spectral power) are negatively correlated with changes in ejection fraction (EF) and indexed stroke volume (iSV), and positively correlated with changes in indexed end systolic volume (iESV). The inverse is true for changes in nLF spectral power (hypothesised to be a marker of sympathetic nerve activity), with a positive correlation versus the changes in EF and iSV and a negative correlation versus

the change in iESV. The change in the LF/HF ratio correlated with the change in iSV but not with a decrease in EF (see Table 5-15). Thus, a decrease in cardiac SNA and an increase in vagal tone were associated with a fall in iSV, and by some measures, a reduction in EF. There were no significant correlations between changes in any of the HRV parameters and any measures of myocardial strain or strain rate as assessed by CMR at 6 months post-RDN.

		EF (%)	LVM (g)	iLVM (g/m2)	iEDV (ml/m2)	iESV (ml/m2)	iSV (ml/m2)
SDNN	R	-0.73	0.23	0.20	0.08	0.60	-0.82
	P	0.001	0.39	0.45	0.76	0.01	0.0001
RMSSD	R	-0.76	-0.05	-0.12	-0.15	0.58	-0.86
	P	0.0007	0.86	0.65	0.57	0.02	< 0.0001
NN50	R	-0.49	-0.02	-0.28	-0.16	0.43	-0.70
	P	0.05	0.94	0.29	0.55	0.10	0.002
pNN50	R	-0.53	-0.03	-0.28	-0.17	0.45	-0.74
	P	0.04	0.92	0.29	0.53	0.08	0.001
Total power	R	-0.69	0.03	0.10	0.05	0.59	-0.57
	P	0.003	0.92	0.71	0.85	0.02	0.02
VLF	R	-0.29	0.34	0.36	0.28	0.31	-0.10
	P	0.28	0.19	0.17	0.30	0.24	0.72
LF	R	-0.31	-0.09	0.09	0.03	0.18	-0.19
	P	0.24	0.74	0.74	0.91	0.50	0.49
nLF	R	0.57	-0.24	-0.22	-0.17	-0.53	0.59
	P	0.020	0.37	0.41	0.54	0.03	0.02
HF	R	-0.77	-0.13	-0.16	-0.21	0.53	-0.90
	P	0.0005	0.63	0.54	0.42	0.03	< 0.0001
nHF	R	-0.22	0.14	0.11	0.07	0.23	-0.25
	P	0.42	0.61	0.69	0.78	0.40	0.35
LF/HF	R	0.29	0.06	0.18	0.16	-0.25	0.61
	P	0.27	0.83	0.51	0.56	0.35	0.01

Table 5-15. Relationships between changes in heart rate variability (HRV) parameters and changes in left ventricular ejection fraction (EF) and volumetric parameters, assessed 6 months after renal denervation.

Data shown (n=16) for Pearson's or Spearman's rank correlations (R) as appropriate based on approximation to normal distribution. Level of significant shown (p), with $p<0.05$ taken to indicate significance. SDNN; standard deviation of differences between successive NN (normal to normal) intervals, RMSSD; square root of the mean squared differences of successive NN intervals, NN50; number of interval differences of successive NN intervals measuring >50 ms, pNN50; NN50 count as a percentage of the total number of all NN intervals, VLF; very low frequency, LF; low frequency, nLF; normalised low frequency, HF; high frequency, nHF; normalised high frequency, LVM; left ventricular mass, iEDV; indexed end diastolic volume, iESV; indexed end systolic

volume, iSV; indexed stroke volume. All indexed values are indexed to body surface area.

Aortic distensibility was not correlated with any of the HRV spectral frequency parameters at baseline, however, baseline aortic distensibility did correlate with baseline NN50 (n=14, R=0.75, p=0.002) and baseline pNN50 (n=14, R=0.70, p=0.005).

There were no significant correlations between the change in aortic distensibility at 6 months post-RDN and the changes in any of the HRV parameters at 6 months after denervation. However, when looking at changes in NN50 and pNN50, which correlated with aortic distensibility at baseline, there was a trend towards a positive correlation (n=13, R=0.47, p=0.11 and R=0.48, p=0.09, respectively), suggesting that aortic distensibility may improve with increasing vagal tone. There was also a trend towards a correlation between the change aortic distensibility and the change in LF/HF at 6 months post-RDN (n=13, R=-0.53, p=0.06); this inverse trend may suggest that as sympathetic tone decreases (or vagal tone increases), aortic distensibility increases, and in thus consistent with the trend in NN50 and pNN50 data above.

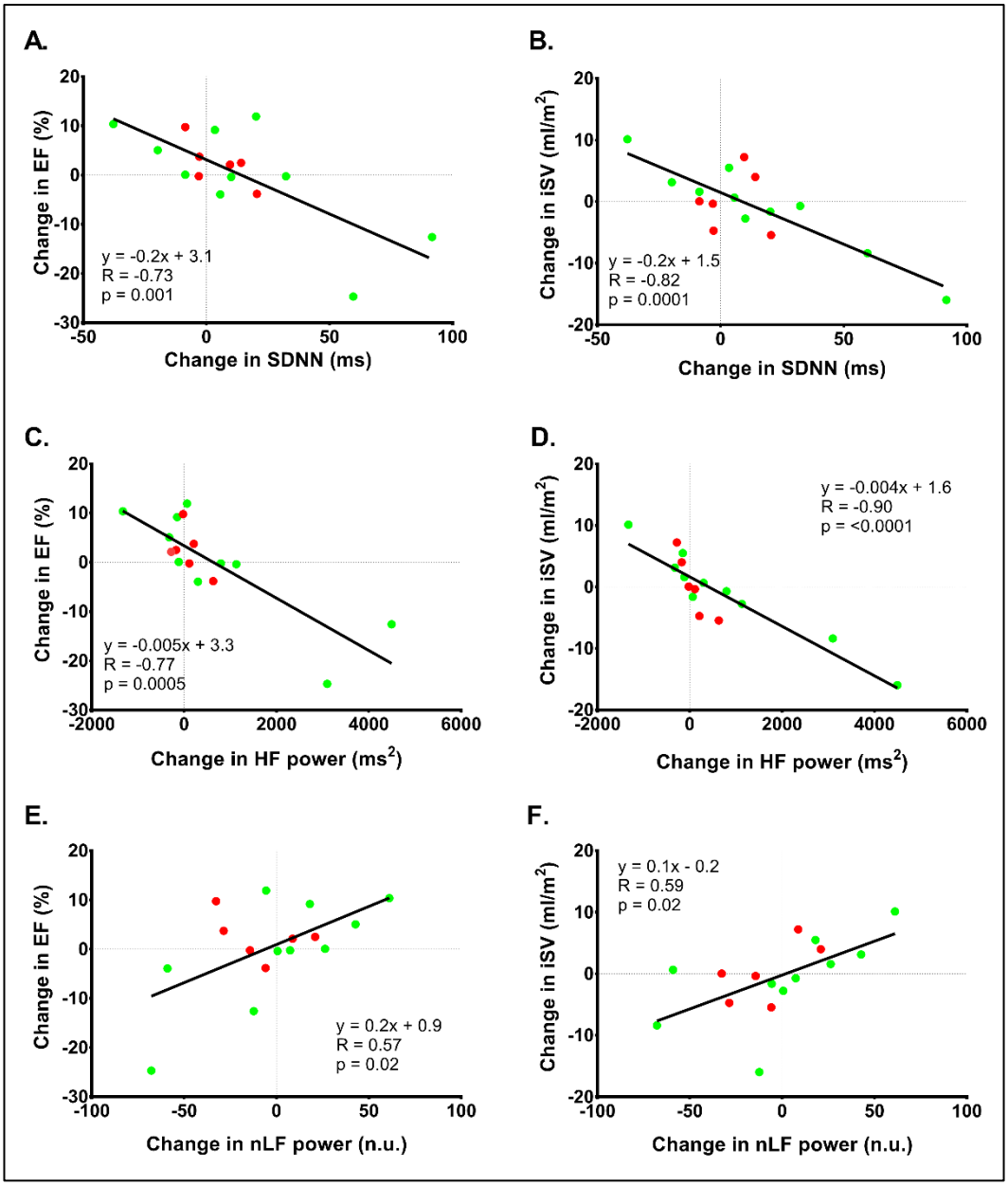


Figure 5-23. Correlations between changes in selected HRV parameters and changes in ejection fraction (EF) and indexed stroke volume (iSV) at 6 months after renal denervation.

Data for RDN BP-responders (reduction in office SBP ≥10 mmHg at 6 months post-RDN) shown in green, data for RDN BP non-responders shown in RDN. Changes in markers of vagal tone (SDNN and HF spectral power) are negatively correlated with changes in EF and iSV, and the inverse is true for changes in nLF spectral power (a marker of sympathetic nerve activity (SNA)). Thus, a decrease in cardiac SNA and an increase in vagal tone were associated with reductions in iSV and EF. SDNN; standard deviation of differences between successive normal to normal intervals, nLF; normalised low frequency, HF; high frequency.

5.3.4 Discussion

5.3.4.1 No overall change in MSNA following renal denervation

In this small study, with microneurography data from 14 patients, there was no reduction in MSNA following RDN, furthermore, there was no correlation between the change in office SBP and the change in MSNA at 6 months post RDN, and no difference in MSNA between RDN BP responders and BP non-responders at any study time-point. These findings are contrary to our hypotheses that RDN would result in a reduction in SNA and that any BP reduction following RDN would be associated with a reduction in MSNA and indicate that changes in BP following RDN are independent of MSNA.

Review of the individual plots of SBP and MSNA data shown in Figure 5-17 would suggest that it is an over simplification to consider these data on a cohort basis. In some individuals (e.g. patients 1, 4, 8, 10, 11 and 12) there is a clear temporal relationship between the changes in BP and MSNA following RDN, which might indicate a mechanistic interaction between these two parameters. In contrast, in other participants (e.g. patients 5, 6 and 7) BP and MSNA appear unrelated (or even inversely related) after denervation.

These findings do not support the data published by Esler's group, who initially developed clinical renal denervation (Schlaich, Sobotka et al. 2009). In the first published case of endovascular renal denervation, Schlaich at al. described a patient in which BP was successfully reduced from 161/107 mm Hg at baseline, to 141/90 mm Hg at 30 days and to 127/81 mm Hg at 12 months (Schlaich, Sobotka et al. 2009). In this case, whole-body noradrenaline (NA) spillover was reduced by 42%, with a reduction in organ specific NA spillover of 48% from the left kidney and 75% from the right kidney. Furthermore, elevated baseline MSNA returned to normal levels (56 bursts/min at baseline, 19 bursts/min at 12 months), cardiac baroreflex sensitivity improved and there was a reduction in left ventricular mass following RDN (Schlaich, Sobotka et al. 2009).

In Symplicity HTN-1, renal NA spillover was assessed in a subgroup of 10 patients and was reduced by 47%, with a concomitant BP reduction of 22/12 mmHg, following RDN (Krum, Schlaich et al. 2009). The data for a reduction in MSNA following RDN have been contradictory. Several studies have reported a reduction in MSNA following RDN (Hering, Lambert et al. 2013, Hering, Lambert et al. 2013, Grassi, Seravalle et al. 2015, Hering, Marusic et al. 2016, Seravalle, D'Arrigo et al. 2017, Tsioufis, Dimitriadis et al. 2017), including sustained reductions in MSNA out to 12 months post-RDN (Hering, Marusic et al. 2014). In the latter study, baseline MSNA was 51±11 bursts/min (Hering, Marusic et al. 2014), which is approximately 2- to 3-fold higher than the level observed in age-matched, healthy controls (Narkiewicz, Phillips et al. 2005), reducing by -6 ± 11 bursts/min ($p<0.01$) at 12-month follow-up (Hering, Marusic et al. 2014). Seravalle et al. reported a related reduction in MSNA and BP using a time-integrated approach (Seravalle, D'Arrigo et al. 2017). In contrast, Grassi et al. reported a significant reduction in MSNA following RDN, which was independent of the preceding reduction in SBP (n=15) (Grassi, Seravalle et al. 2015). Hering et al. described a reduction in both multiunit MSNA and single-unit MSNA (including firing rates of individual muscle vasoconstrictor fibres, firing probability, and multiple firing incidence of single units within a cardiac cycle), interestingly, once again there was no correlation between

changes in both multiunit MSNA and single-unit MSNA and BP following RDN (Hering, Lambert et al. 2013).

There are also data reporting no reduction in BP or MSNA following RDN, and the outcomes above have not been consistently reproducible (Brinkmann, Heusser et al. 2012, Hart, McBryde et al. 2013, Vink, Verloop et al. 2014, Tank, Heusser et al. 2015). Hart et al. reported autonomic data before and after RDN in 7 patients (4 of which are participants in this study); these early data showed no overall change in MSNA at 1 or 6 months following RDN, but MSNA did fall by >10% in 4/7 patients, although these changes in MSNA did not correlate with any change in SBP (Hart, McBryde et al. 2013). Brinkmann et al. showed no change in supine blood pressure, resting MSNA (pre-, 34±2 bursts/min; post-, 32±3 bursts/min, p=0.6) or heart rate variability in a small cohort of 12 patients following RDN (Brinkmann, Heusser et al. 2012), although this study was criticised for the inclusion of patients with moderate hypertension and lower resting MSNA than seen in the Symplicity cohorts. In the DREAMS (Denervation of the Renal Arteries in Metabolic Syndrome) study, investigators reported a significant 6/5 mmHg reduction in mean 24hr BP, but no change in MSNA (n=29); MSNA was recorded in a subset of patients and did not differ between baseline and 6 month follow-up (74 ± 48 vs 75 ± 23 bursts/100HB, respectively, p=0.80) (Verloop, Spiering et al. 2015).

Baseline MSNA incidence was inversely correlated with baseline heart rate (R=-0.63, p=0.02), but did not correlate with baseline MSNA frequency (R=-0.14, p=0.63, see Figure 5-14). This may suggest that if a patient has fewer heart beats in a minute, a greater proportion of the heart beats seen within that minute is associated with an MSNA burst in order to maintain peripheral vascular tone. Having said this, there was no correlation between baseline MSNA and baseline estimated TPR. There was no correlation between the change in resting heart rate and the change in either MSNA incidence or frequency, at 6 months post-RDN, implying that SNA to the peripheral vasculature is independently regulated from the sympathovagal balance controlling the sinus node. There was also no correlation between the change in MSNA and the change in estimated TPR at 6 months post-RDN, however the estimate of TPR used in this analysis was based on cardiac output from the CMR data and brachial BP which were not measured simultaneously, and therefore should be interpreted with caution.

5.3.4.2 Relationship between blood pressure and MSNA

Interestingly, there was an inverse correlation between office SBP and MSNA incidence at baseline (Figure 5-14), when it could have been expected that individuals with a higher BP would have higher MSNA. This concept is based on evidence showing that MSNA is elevated in hypertension (Yamada, Miyajima et al. 1989), and that, in subjects aged 40 years and above, MSNA increases with increases mean arterial pressure (Narkiewicz, Phillips et al. 2005). However, this conclusion makes some assumptions, firstly that high SNA is responsible for high BP, rather than other mechanisms such as the renal – body fluid hypothesis or endocrine factors (see Section 2.1.1.1). Secondly, for MSNA to be persistently raised in the face of raised BP, there is the implication that the baroreflex is not operating effectively, since any increase in arterial pressure should activate the sympathoinhibitory baroreflex, and thus reduce MSNA back towards baseline levels (Guyton and Hall 1996). The effect of RDN on baroreflex sensitivity is discussed further in Section 5.4.1. Finally, not all subjects were aged >40years.

Based on categories for normal and raised MSNA as shown in Table 5-16 (Narkiewicz, Phillips et al. 2005, Hering, Marusic et al. 2014), at baseline, 4/14 (29%) participants had normal range MSNA frequency (3 women), 3/14 (21%) had mildly elevated MSNA frequency, 5/14 (36%) had moderately elevated MSNA frequency, and 2/14 (14%) had extremely elevated MSNA frequency. This would suggest differences between our population and the population in the Hering et al. study in which 33/35 (>90%) of patients had MSNA above the normal range for their age and gender (Hering, Marusic et al. 2014). At 6 months post-RDN, 3/12 (25%) participants had normal range MSNA frequency (3 women), 2/12 (17%) had mildly elevated MSNA frequency, 5/12 (42%) had moderately elevated MSNA frequency, and 2/12 (17%) had highly elevated MSNA. MSNA frequency did not normalise following RDN in any of the participants with raised MSNA at baseline; those with normal MSNA at baseline had normal range MSNA at 6 months post-RDN.

Age, y	20–29	30–39	40–49	50–59	≥60	≥70
Men						
Normal	≤22*	≤26*	≤27*	≤31*	≤36*	≤40
Mildly elevated	23–29	27–33	28–34	32–39	37–42	41–44
Moderately elevated	30–36	34–40	35–41	40–48	43–48	45–48
Highly elevated	37–43	41–47	42–48	49–58	49–54	49–52
Extremely elevated	≥44	≥48	≥49	≥59	≥55	≥53
Women						
Normal	≤14*	≤23*	≤30*	≤32*	≤42*	≤44
Mildly elevated	15–20	24–30	31–38	33–41	43–48	45–48
Moderately elevated	21–28	30–37	39–46	42–52	49–55	49–53
Highly elevated	29–38	38–44	49–54	53–61	56–64	54–59
Extremely elevated	≥39	≥45	≥55	≥62	≥65	≥60

Table 5-16. Novel categories of abnormal resting muscle sympathetic nerve activity (MSNA) in men and women according to age.
Table from Hering et al. (Hering, Marusic et al. 2014). MSNA values are for MSNA frequency expressed in burst/min. *Previously demonstrated MSNA values for healthy male and female subjects described by Narkiewicz et al. (Narkiewicz, Phillips et al. 2005).

MSNA is not related to BP below the age of 40 years, particularly in pre-menopausal women (Narkiewicz, Phillips et al. 2005), and it is relevant to note that the inverse correlation between baseline SBP and baseline MSNA seen in this cohort disappears when the five premenopausal women are removed from the analysis (R=-0.38, p=0.31); several of these individuals had particularly high baseline SBP relative to their lower level MSNA (e.g. normal range MSNA in three of these women). The lack of correlation between SBP and MSNA at baseline in these younger patients may, in part, explain the lack of a correlation between MSNA and BP reduction post-RDN in this study. However, the pre-menopausal participants were patients number 2, 4, 10, 11 and 15; qualitative review of the data for these individuals as shown in Figure 5-17 again suggests that this theory may be an over simplification, since the patterns in SBP and MSNA data for patients 4, 10 and 11 would indicate a temporal relationship between BP and SNA following RDN in these individuals.

Our patients had lower baseline MSNA than those in the Hering et al. study (38 ± 3 bursts/min or 60 ± 6 bursts/100 heartbeats vs 51 ± 11 bursts/min or 80±16 bursts/100 heartbeats, respectively (Hering, Marusic et al. 2014)), and this may also contribute to the difference in outcome with respect to a change in MSNA post-RDN between these studies. Our data are more similar to that of Brinkmann et al. with a study population with a similar, lower, level of baseline MSNA (34±2 bursts/min) (Brinkmann, Heusser et al. 2012).

In this study we present data on MSNA, which is a marker of sympathetic drive to the peripheral vasculature, and it is possible that this means that we have not been able to quantify significant changes in organ specific sympathetic nerve active, as is assessed by measures such as renal NA spillover. Our pilot study is also under-powered, and data from a broader population treated with RDN is required to establish any clear associations between gender, age and MSNA in the context of renal nerve ablation.

5.3.4.3 No change in heart rate variability following renal denervation

HRV has the advantage over MSNA of being relatively easily assessed from a resting ECG recording, with data obtained at baseline in 17/18 participants in this study. However, HRV is only a surrogate marker for changes in autonomic balance, rather than a direct measure of SNA or vagal tone (Hedman, Hartikainen et al. 1995). In this study, there were no significant correlations between any of the HRV parameters at baseline and either office SBP, resting HR or MSNA, prior to RDN. Following treatment, none of the HRV parameters changed significantly over the course of the study, and there were no correlations between the change in office SBP and the changes in any of the HRV parameters at 6 months after the procedure

Prior to RDN, there were no differences in any of the HRV parameters between RDN responders and non-responders. At 6 months post-RDN, there was a significant difference in nHF measures between responders and non-responders, (nHF 34 ± 5 n.u. vs 57 ± 7 n.u. respectively ($p=0.02$)). Overall mean nHF at baseline was 39 ± 5 n.u.; this would suggest that non-responders had had a relative increase in nHF, and therefore vagal tone, following RDN. This is difficult to rationalise if, by definition, these individuals have failed to respond to the intervention, furthermore, there was no significant change in nHF over the course of the study amongst either responders or non-responders, and therefore any inferences from this finding should be interpreted with caution. The LF/HF was also borderline higher in responders as compared with non-responders at 6-months, with data indicating primarily an increase in vagal tone and/or a decrease in cardiac SNA following RDN amongst the non-responders, although neither response group had a significant change in LF/HF ratio over the course of the study. This pattern would go against our hypothesis that response to RDN would be associated with a reduction in sympathetic tone. It may be that these data are unclear due to the small sample size in this study, or that HRV parameters are a poor marker for SNA in this context, focussing on cardiac changes in cardiac sympathovagal balance, rather than SNA to the peripheral vasculature or kidney.

When looking at the effect of RDN on HRV, published data provide little support for a reduction in SNA following RDN, and are consistent with our results. Brinkmann et al. reported no change in HRV following RDN (Brinkmann, Heusser et al. 2012). In the

DREAMS study, there was a significant reduction in BP, but no change in HRV frequency parameters (n=26) (Verloop, Spiering et al. 2015). The ReSET trial, a sham-controlled trial of RDN, reported a reduction in BP following RDN, but as with Symplicity HTN-3, this change did not differ from the sham control group (Peters, Mathiassen et al. 2017). This study showed no significant change in HRV parameters in comparison to sham participants following RDN, and that HRV parameters were not predictive of the BP response to RDN (Peters, Mathiassen et al. 2017). In contrast, Tsioufis et al. reported improvements in both time- and frequency-domain indices of HRV out to 6 months post-RDN (Tsioufis, Papademetriou et al. 2014). Overall, these findings may suggest that RDN has little effect on HRV, and therefore cardiac SNA, however, it may be that HRV is a poor marker for cardiac SNA, or the modulation of SNA affecting other organs, in this context (Peters, Mathiassen et al. 2017).

A limitation of MSNA as a measure of sympathetic activity is that it records sympathetic drive to the vasculature in the muscle bed only. SNA control is organ specific, and differentially controlled (Esler, Jennings et al. 1984, Osborn and Fink 2010, May, Howard Florey Institute et al. 2017), and therefore there has been interest as to whether RDN impacts renal and cardiac SNA. NA spillover can quantify organ specific SNA but is invasive and requires specialist resources and operators; the sub-study in Symplicity HTN-1 described above, did however, demonstrate a reduction in renal SNA following RDN (Krum, Schlaich et al. 2009). Booth et al. assessed the effect of RDN on cardiac SNA through direct measurement in an ovine model, and showed no change in cardiac SNA post-RDN, although there was a leftward shift in in cardiac baroreflex sensitivity (Booth, Schlaich et al. 2015). Donazzan et al. used I-123-metaiodobenzylguanidine (MIBG) imaging to assess cardiac sympathetic innervation and cardiac sympathetic activity. MIBG is a radiopharmaceutical agent sharing the uptake into sympathetic nerve with NA. Analysis of global MIBG uptake, and thus cardiac sympathetic innervation, was quantified as the heart-to-mediastinum ratio (upper mediastinal reading taken as null) and the difference in tracer uptake/retention between early and late images (washout ratio) quantified cardiac sympathetic activity. Cardiac sympathetic innervation remained unchanged before and after the procedure, but cardiac sympathetic activity was reduced after RDN, independent of the BP outcome (Donazzan, Mahfoud et al. 2016). Overall, there is no consistent evidence to support a reduction in cardiac SNA following RDN, which is interesting given reproducible reductions in left ventricular hypertrophy and improvements in cardiac function (Brandt, Reda et al. 2012, Bruno and Taddei 2014, Mahfoud, Urban et al. 2014, Schirmer, Sayed et al. 2014, Di Daniele, Rovella et al. 2015, Lu, Wang et al. 2016), including those seen in this cohort (see Table 5-6).

5.3.4.4 Does sympathomodulation impact target organ damage after renal denervation?

5.3.4.4.1 Sympathetic nerve activity and changes in left ventricular mass and function following renal denervation

At baseline, whilst the correlation between MSNA and indexed LVM did not achieve significance (R=0.44, p=0.12), there were significant correlations between MSNA and measures of LV strain. Participants with higher MSNA may trend towards increased LVM index, but had significant correlations indicating impaired/reduced peak radial strain (thickening) and peak circumferential and longitudinal strain (shortening), and similarly

reduced peak systolic and diastolic radial, circumferential and longitudinal strain rates at higher levels of MSNA (see Figure 5-19). This observation is particularly interesting since MSNA was inversely correlated with SBP at baseline (Figure 5-14), and although SBP did not correlate with any of the CMR parameters at baseline, the reduction in office SBP was associated with an improvement (reduction) in LV mass and LV mass index, and improvements in peak radial (increased thickening) and peak circumferential (increased shortening) strain (see Figure 5-13). If raised MSNA was associated with impaired myocardial strain, and if raised MSNA was also associated with lower SBP, then impaired myocardial strain might have been expected to be associated with lower SBP. This final statement was not born out by our baseline data, but would seem counterintuitive, and is not supported by the concordant improvements in SBP and myocardial strain parameters following RDN. This disparity may relate to the fact that the study is underpowered, but may also be impacted by the premenopausal women in the study in whom no relationship between SNA and BP could have been expected (see Section 5.3.4.2) (Narkiewicz, Phillips et al. 2005). It may also indicate organ specific changes in SNA following RDN.

Whilst the reduction in LVM index following RDN correlated with a reduction in office SBP, there was no correlation between the change in MSNA incidence and the change in LVM index at 6 months post-RDN. There was a significant correlation showing those patients with a decrease in MSNA had a decrease in stroke volume following RDN ($R=0.66$, $p=0.03$), but this was not born out amongst other volumetric and strain parameters aside from a trend for a decrease in MSNA following RDN to be associated with improved (more negative) peak longitudinal strain rate ($R=0.59$, $p=0.05$). These correlations are difficult to interpret since overall MSNA and stroke volume did not change following RDN. If stroke volume decreased in some individuals post RDN, this may be due to a reduction in blood volume, because if both renal and muscle SNA are reduced following RDN, then there would likely be a reduction in sympathetically mediated renin release and Na^+ and water retention (Sobotka, Mahfoud et al. 2011).

As described above, raised baseline MSNA was associated with reduced LV function as assessed by strain parameters, and a trend towards an increase in left ventricular hypertrophy. Hypertensive left ventricular hypertrophy has previously been shown to be associated with increased sympathetic activation (Greenwood, Scott et al. 2001, Schlaich, Kaye et al. 2003, Burns, Sivananthan et al. 2007), but this has not been a universal finding, any may again relate to gender (Best, USA et al. 2018). Interestingly, as described in Section 5.2.4.5, whilst a reduction in LVM index and an improvement in LV function have also been demonstrated previously following RDN, these beneficial effects were independent of reductions in BP (Brandt, Mahfoud et al. 2012, Bruno and Taddei 2014, Doltra, Messroghli et al. 2014, Mahfoud, Urban et al. 2014, Schirmer, Sayed et al. 2014, McLellan, Schlaich et al. 2015, Tsioufis, Papademetriou et al. 2015, Kiuchi, Mion et al. 2016, Tsioufis, Papademetriou et al. 2016). In this instance, it could be hypothesised that the fall in LVM following RDN may relate to cardiac sympathetic withdrawal, rather than reduced BP and cardiac afterload, and therefore contradicts the results of this study. However, the impact of RDN on MSNA has been variable between studies, and the data on any relationship between any change in LVM and MSNA following RDN is limited. More importantly, MSNA is a measure of sympathetic drive to the peripheral vasculature, and may be differentially controlled versus cardiac SNA, and therefore, whilst changes in MSNA could affect peripheral resistance and thus BP and afterload, it

may not be the best measure of sympathetic drive to the myocardium (Esler, Jennings et al. 1984).

HRV parameters may give a better surrogate marker for cardiac sympathovagal balance. At baseline, impaired longitudinal cardiac function was associated with markers for increased cardiac sympathetic tone and cardiac sympathovagal balance (nLF and LF/HF, see Figure 5-22). This pattern is similar to that seen with baseline MSNA which was also related to impaired myocardial strain (see Figure 5-19). At 6 months post RDN, markers indicating a reduction in cardiac SNA and an increase in vagal tone with were associated with a reduction in indexed stroke volume and a reduction in EF, although there were no correlations between changes in any of the HRV parameters and changes in myocardial strain parameters. Although not all of the correlations between changes in measures of HRV and MSNA and changes in cardiac volumetrics and function are in agreement, and no correlation was seen between baseline MSNA and HRV or the changes in MSNA and HRV at 6 months post-RDN, there are several patterns in the data that are consistent between MSNA and HRV parameters, which may suggest similar modulation of cardiac and peripheral SNA by RDN.

Overall, these data would suggest that whilst chronic exposure to raised SNA may contribute to raised LVM index and impaired cardiac function, the improvement in LVM index seen following RDN is independent of SNA, and, given that the change in LVM correlated with a change in SBP in this cohort, the mechanism for this cardiac remodelling is likely to relate to a reduction in afterload, rather than reduced sympathetic tone. The mechanism underlying any potential improvement in cardiac function may be multifactorial. Left ventricular ejection fraction and strain parameters did not significantly improve over the course of this study (although measures of myocardial strain may show a trend towards improvement, see Table 5-6). Improvements in peak radial (increased thickening) and peak circumferential (increased shortening) strain were associated with a reduction in office SBP, but were not correlated with a change in MSNA, suggesting a beneficial effect of decreased afterload rather than sympathoinhibition. MSNA may not reflect cardiac SNA, although changes in some cardiac HRV parameters do appear to demonstrate a similar pattern to changes in MSNA, and therefore, whilst SNA can be differentially controlled between organs, there may be some similarity in their response after RDN. As with the MSNA data, there was no correlation between the change in SBP and the change in HRV parameters, and no correlation between the change in any HRV parameter and change in LV strain at 6 months post-RDN, thus supporting this conclusion.

Schlaich et al. described reductions in BP, MSNA and LVM in the first clinical case report of RDN as a treatment for resistant hypertension (Schlaich, Sobotka et al. 2009), likewise Hoye et al. also report reduced BP, MSNA and LV mass following RDN in a cohort of 9 patients with end-stage kidney disease (Hoye, Wilson et al. 2017). In the latter study, no comment is made about whether there was any relationship between the changes in these primary outcomes measures, although numbers were likely too small to draw firm conclusions. This study presents the largest cohort reporting outcome data for BP, MSNA and LVM following RDN, and although still limited, our data would suggest that the improvement in LVM index, a form of target organ damage, seen following RDN is related to the reduction in SBP (and thus afterload), and is independent of MSNA. These results are contradictory to previously reported studies which reported a reduction in

LVM independent of BP, but do not support the hypothesis that the cardiac remodelling is due to reduced sympathetic activation.

5.3.4.4.2 Sympathetic nerve activity and aortic distensibility following renal denervation

There was no correlation between baseline aortic distensibility and baseline MSNA or any of the baseline HRV spectral frequency parameters. Baseline aortic distensibility did, however, correlate with baseline NN50 and pNN50. Previous research has similarly demonstrated a correlation between impaired HRV, as assessed by SDNN, and increased vascular stiffness as assessed by pulse pressure (Chrysohoou, Skoumas et al. 2013). Data from healthy individuals have shown a correlation between MSNA and vascular stiffness as assessed by pulse wave velocity, with subjects with increased vascular stiffness having higher MSNA (Swierblewska, Hering et al. 2010). Interestingly, young premenopausal women have been shown to have an inverse correlation between MSNA and vascular resistance (Casey, Curry et al. 2011, Harvey, Barnes et al. 2017), which may again, in part explain why our MSNA baseline data do not correlate with baseline aortic distensibility.

There was no correlation between the change in MSNA and either the change in aortic compliance or distensibility at 6 months post-RDN. There were also no significant correlations between the change in aortic distensibility at 6 months post-RDN and the changes in any of the HRV parameters at 6 months after denervation. There was a trend towards a correlation between the change aortic distensibility and the change in LF/HF at 6 months post-RDN, which may suggest that as sympathetic tone decreases (or vagal tone increases), aortic distensibility increases, and would be consistent with the relationship between SNA and vascular stiffness described above.

There is limited available data on the relationship between changes in vascular stiffness and SNA following RDN. Hering et al. report a reduction in augmentation index (a measure of the augmentation of the central arterial pressure waveform due to the reflected wave from the distal vasculature) independent of changes in BP or MSNA, but do not present data on HRV, or cardiac SNA as assessed by other means, in relation to this marker of vascular stiffness (Hering, Lambert et al. 2013). Further research is required to more completely explore this relationship.

5.3.4.5 Study limitations

The small numbers of patients in this study with available MSNA (n=14) and HRV data (n=17), limits the conclusions that can be drawn from what is fundamentally a pilot study, particularly when considering analysis by BP response group.

Microneurography has the limitation of measuring an isolated sympathetic outflow to one vascular bed, and there are many vascular beds, including the renal, cardiac and splanchnic circulations, that cannot be accessed using this technique. Mechanistically, relevant changes in organ specific SNA cannot be excluded from these data, particularly, reductions in cardiac SNA impacting left ventricular hypertrophy, although the quantification of HRV may go some way to address this.

In this study, we have assessed multiunit MSNA. Single unit measurement of sympathetic neurons was not performed, and may give additional information about the

frequency of post-ganglionic neuron firing, the probability of neuron firing in any particular multi-unit burst, and the occurrence of multiple unit firing in a single burst of activity to a vascular bed, providing more detailed information about the modulation of SNA (Macefield, Wallin et al. 1994, Hering, Lambert et al. 2013).

The HRV analyses performed in this study are based on a short-term, 5-minute, ECG recording, more robust data may be obtained from a full 24hr recording, giving a more valid assessment of lower frequency HRV components (1996). Data from eight follow-up visits could not be included in the analyses due to an inadequate 5-minute ECG recording, either due to an erratic ECG baseline, or excess ectopic activity. HRV is not a direct measure of sympathetic nerve activity and as such, HRV parameters can only be interpreted as indices of autonomic activity; the lack of correlation between MSNA measures and HRV parameters in this study would suggest that this may be the case.

5.3.5 Conclusions

In this cohort there was no reduction in SNA as measured by either microneurographic MSNA or time- and frequency-domain HRV parameters following renal denervation. These findings do not support the hypothesis that RDN reduces BP through a reduction in systemic SNA by disrupting the central input from excess renal afferent nerve activity. Given that BP did fall following RDN, since MSNA remained unchanged, this means that baroreflex sensitivity must have reset, because with an intact baroreflex, a fall in BP would result in an increase in MSNA, and thus TPR in order to return BP to baseline levels (Lohmeier and Iliescu 2015). Furthermore, in this, the largest cohort to date reporting outcome data for BP, MSNA and LVM following RDN, our data suggest that the improvement in LVM seen following RDN is also independent of any change in MSNA or cardiac SNA as assessed by HRV. There was no correlation between baseline MSNA or HRV and the BP response to RDN, indicating that RDN is not acting through a reduction in renal efferent SNA, although this cannot be excluded by these data since microneurography does not provide a measure of renal specific SNA or SNA to other muscle or non-muscle vascular beds. The mechanism underlying the antihypertensive and positive cardiac remodelling effects of RDN remain unclear, but I will go on to explore the effect on baroreflex sensitivity in the next chapter.

The lack of correlation between MSNA measures and HRV indices supports a differential regulation of muscle and cardiac SNA outflows, and suggests that these markers are unlikely to provide a robust marker for procedural efficacy, patient selection or mechanistic investigations in future studies of RDN.

Finally, and intriguingly, when the data were reviewed at the level of the individual patient, there did seem to be a temporal relationship between MSNA and office SBP following RDN in some of the patients studied, and the conclusions drawn on a cohort basis may be an oversimplification. These are pilot data and as such should guide future research but are not suitable for extrapolation to the wider population. Further research is required into the mechanisms underlying RDN and the relationship between RDN and MSNA, particularly in light of the positive preliminary data from the SPYRAL HTN studies which are likely to reignite interest in the technique (Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018).

5.4 Autonomic modulation following renal denervation

The initial hypotheses for the mechanism underlying the antihypertensive effect of RDN focussed on a reduction in sympathetic nerve activity, either through reducing the efferent renal SNA controlling the kidney, or through a reduction in systemic SNA due to decreased central sympathoexcitation through a reduction in feedback from the afferent renal nerves (Sobotka, Mahfoud et al. 2011). The autonomic control of blood pressure and potential interactions with renal nerve ablation, which are likely to differ between individuals, were reviewed in Sections 2.1.2 and 2.3.1 are clearly much more complex than this, with inputs from a range of neurohormonal mechanisms, including feedback from baroreceptors, peripheral chemoreceptors and interaction with systemic inflammation and cerebral perfusion. In this section, I will assess the impact of renal denervation on a range of physiological systems known to be involved in the regulation of blood pressure.

5.4.1 Baroreflex Sensitivity

5.4.1.1 Introduction

Baroreceptors are located in the aorta and carotid sinus. Distention of these vessels due to increased intra-arterial pressure activates the baroreceptors which have a sympathoinhibitory effect, thereby reducing vascular tone and hence, arterial pressure. The gain in this reflex mechanism is known as *sympathetic* (vasomotor) baroreflex sensitivity (sBRS). Sympathetic efferent neurones also innervate the heart, and their activation increases myocardial contractility and heart rate (Charkoudian and Rabbitts 2009). Baroreceptor activation will also, therefore, cause a reflex reduction in heart rate, and the sensitivity of this feedback loop is known as *cardiac* baroreflex sensitivity (cBRS) which is affected by alterations in the balance between sympathetic and vagal nerve input to the sinus node. sBRS and cBRS have both be shown to be in impaired in hypertension with data also suggesting that the impairment in BRS may precede the onset of hypertension (Yamada, Miyajima et al. 1988, Minami, Imai et al. 1989, Matsukawa, Gotoh et al. 1991, Matsukawa, Gotoh et al. 1991, Hesse, Charkoudian et al. 2007, Honzikova and Fiser 2009). Reduced BRS is an independent indicator for all-cause mortality and of cardiovascular morbidity in hypertensive patients (and in other conditions of sympathetic over activity) (Johansson, Gao et al. 2007, Ormezzano, Cracowski et al. 2008), furthermore, positive antihypertensive effects have also been reported following the use of baroreceptor activation therapy (see Section 2.3.8.1). In light of these factors, it is important to consider the impact of renal denervation on BRS as a potential mechanism for BP modulation and/or an improvement in target organ damage, including hypertensive heart disease. Importantly, as reported in Section 5.3.3.1, a reduction in office systolic blood pressure (SBP) following RDN was not associated with a change in muscle sympathetic nerve activity (MSNA), and given the sympathetic baroreflex pathway described above, if SNA remains unchanged after RDN despite a reduction in SBP, then it would be predicted that there has been an alteration

in either BRS or the operating point of the baroreflex curve, re-setting to a lower BP range.

In this study, we have primarily assessed *spontaneous* baroreflex sensitivity through evaluation of MSNA and the heart rate response to changes in BP for the measurement of spontaneous sBRS and spontaneous cBRS baroreflex gain. In a subset of 10 patients, a modified Oxford protocol was used to dynamically assess the sBRS and cBRS over a wider BP range through use of *pharmacological* vasodilation and vasoconstriction.

Most studies in this field report sBRS as assessed by the threshold method (BRST) which looks at the probability of any particular cardiac cycle being associated with a MSNA burst; at higher BP, the baroreflex is activated and MSNA is suppressed meaning the cycles with a higher diastolic BP (sBRS is more strongly associated with DBP than SBP) are less likely to be associated with a burst of MSNA (Kienbaum, Karlssonn et al. 2001, Hart, Joyner et al. 2010). A downfall of the BRST method is that it implicitly depends on the level of sympathetic nerve activity (Fadel 2011, Hart, Wallin et al. 2011). In particular, if MSNA burst incidence is high (e.g. >80 bursts/100 heart beats), baroreflex sensitivity is quantified as low, because most DBPs (whether high or low) fail to meet the *threshold* to inhibit the occurrence of a burst (see Figure 5-24). In this instance, changes in DBP may still modulate MSNA burst *strength* (i.e. MSNA burst amplitude or area), which are quantities of sympathetic output not captured by the threshold method. Consequently, in conditions where MSNA burst incidence is high, such as hypertension, the threshold method may indicate poor baroreflex sensitivity since there is little variation in the percentage of BPs in a 1mmHg bin associated with a burst, giving a flattened sBRS slope (Fadel 2011, Hart, Wallin et al. 2011). Whether this reduction in sensitivity is a genuine impairment of the sympathetic baroreflex, or simply due to the method's dependence on MSNA burst incidence, is not clear, but given this observation, we have also calculated sBRS using an area method (BRSA) in which mean burst area is associated with the average diastolic blood pressure (DBP) across the range of DBP recorded. This method is similar to the segregated signal-averaging method designed by Halliwill (Halliwill 2000), where the area under the neurogram is averaged for each cardiac cycle and then binned in 3 mmHg DBP bins.

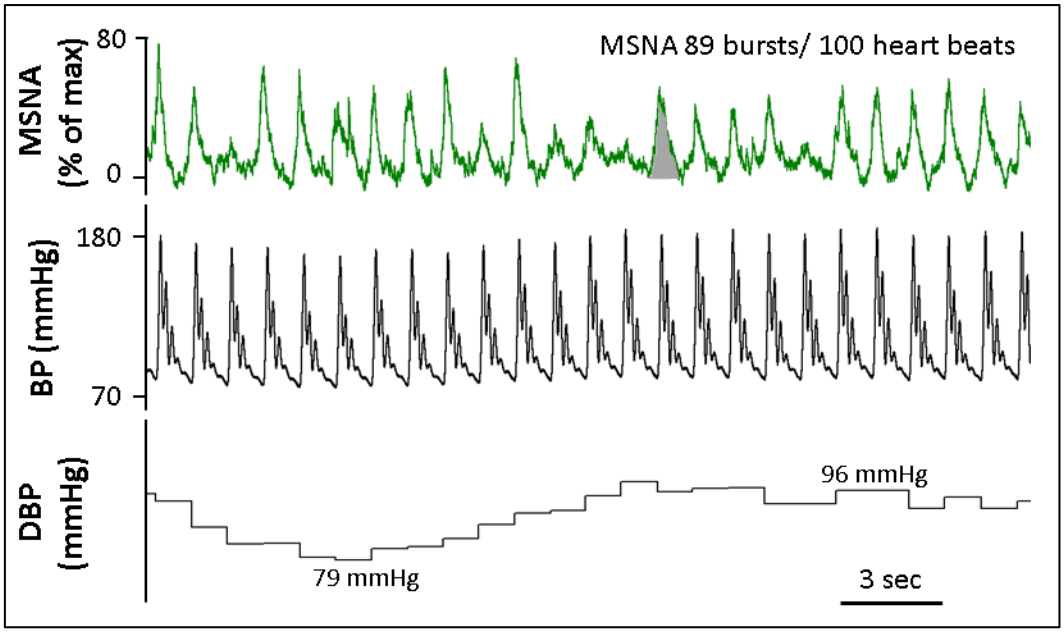


Figure 5-24. Example of the effect of spontaneous fluctuations in diastolic blood pressure (DBP) on muscle sympathetic nerve activity (MSNA) in a patient with high MSNA.
This figure illustrates the potential difficulties in assessing sympathetic baroreflex sensitivity (sBRS) using the threshold method in patients with high MSNA. As DBP rises there is still an MSNA burst associated with nearly every cardiac cycle which would equate to seriously impaired sBRS using the threshold method. However, as DBP rises there is a reduction in burst amplitude and area (see example of burst area shaded in grey) which suggest preserved sBRS as quantified by an area method.

5.4.1.2 Methods

Continuous, simultaneous recordings of RR interval (3-lead ECG), BP (Finometer) and MSNA were made over a 5-10-minute period with subjects laid semi-supine at quite rest as previously described in Sections 4.3.5 and 4.3.6.

5.4.1.2.1 Analysis of sympathetic baroreflex sensitivity

For the analysis of BRST, the DBPs for each cardiac cycle during the recording period were grouped into 1mmHg BP bins; the percentage of cardiac cycles associated with an MSNA burst in each DBP bin was then calculated and associated with the mean DBP in the corresponding bin. The slope of the relationship between the mean DBP and % MSNA for each DBP bin was calculated using linear regression (weighted for the number of bursts in each bin) and quantifies the sympathetic sBRST (custom written script by Dr L. Briant; Matlab, The MathWorks, Natick, MA; for example see Figure 5-25) (Hart, Joyner et al. 2010). The slope of this relationship has been previously shown to agree with the pharmacological sBRS calculated during a modified Oxford baroreflex test (Hart, Joyner et al. 2010).

For the analysis of BRSA, the peak, beginning and end of each MSNA burst were marked in a data acquisition programme (Spike2, Cambridge Electronic Design Ltd, Cambridge, UK); burst peaks were identified using a custom-written script (Dr E. Hart, University of Bristol, UK) and confirmed by visual inspection (Dr A. Burchell), the beginning and end of each burst were marked manually (Dr A. Burchell). The area of the burst was then calculated as the integral of MSNA between the beginning and end of the burst (custom script, Dr L. Briant, University of Bristol, UK; MATLAB, The MathWorks, Natick, MA, USA). Each MSNA burst area (units were AU·s) was normalised to the largest burst area in that recording and represented as a percentage of it. To avoid skewing of area measures by signal drift, the area measured was calculated in the region bounded below MSNA by a constant line intersecting the beginning/end of the MSNA burst. Each cardiac cycle was then associated with an MSNA burst area (=0 AU if no burst occurred in that cycle). For each DBP (1mmHg bins), the average MSNA burst area for all cardiac cycles with that DBP were then calculated. This average area was plotted as a function of DBP, and a weighted (number of MSNA bursts with that DBP) linear regression was fitted to the linear part of the data, yielding the measure of sBRSA.

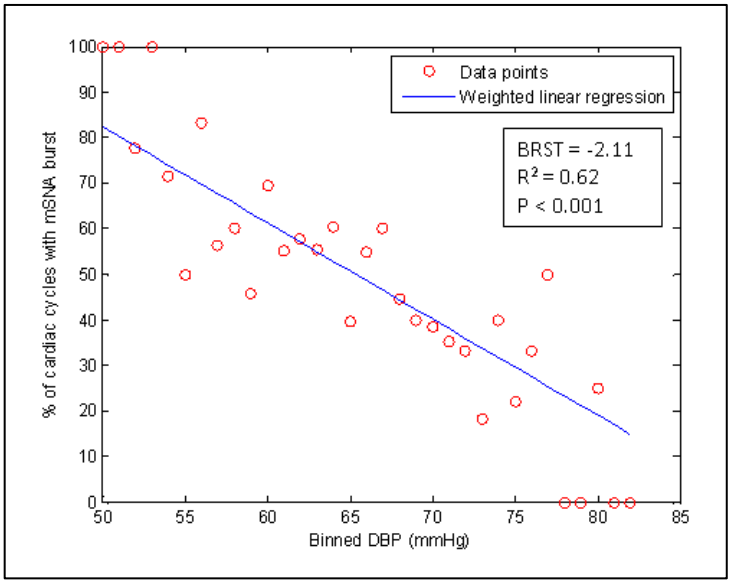


Figure 5-25. Example showing the calculation of sympathetic baroreflex sensitivity using the threshold method.
The graph shows the relationship between the percentage of cycles within a 1 mmHg diastolic blood pressure (DBP) bin associated with a burst of muscle sympathetic nerve activity (MSNA) versus the mean DBP for that bin. The slope of the weighted linear regression gives the sympathetic baroreflex sensitivity by the threshold method (BRST). Data shown are baseline data, pre-RDN, for patient number 7 in this study, who had a MSNA incidence of 53 bursts/100 heart beats.

Previous studies have also demonstrated a difference in the MSNA response to rising versus falling DBP; Studinger et al. indicate that in young healthy adults, the sympathetic baroreflex is more responsive to increasing rather than decreasing pressures (Studinger, Goldstein et al. 2007, Dutoit, Hart et al. 2010). In light of this, both BRST and BRSA data are presented for total, rising and falling DBP as has been described previously (Hart, Wallin et al. 2011, Hart, McBryde et al. 2013). Specifically, DBP were identified that were

preceded by a single DBP that was lower (or higher), which were considered to be rising (or falling, respectively) DBP.

5.4.1.2.2 Modified Oxford Protocol

Spontaneous sympathetic (and cardiac) BRS data were assessed in all patients with available data. In a subset of 10 patients, pharmacological BRS was quantified using the modified Oxford protocol. Vasodilator (sodium nitroprusside) and vasoconstrictor (phenylephrine) drugs were used to artificial manipulate BP over a wider range than that observed in the measurement of spontaneous, resting BRS. The modified Oxford technique is the gold standard measurement of baroreflex function (Rudas, Crossman et al. 1999) and has been used to measure baroreflex sensitivity in many different sub-sets of patients, from hypertensives (Laterza, de Matos et al. 2007) to diabetic patients (Eckberg, Harkins et al. 1986).

The Modified Oxford technique was performed as previously described (Charkoudian, Martin et al. 2004, Hart, Joyner et al. 2010). Specifically, after a 5-10-minute baseline period, 100 µg of sodium nitroprusside was given intravenously as a bolus to lower arterial pressure, followed 1 minute later by 150 µg of phenylephrine to raise arterial pressure to baseline levels. Recovery data were then collected for an additional 2 minutes. Please note, the aim was to reduce SBP by 10-20 mmHg and then return SBP to resting levels, some patients were sensitive to the vasoactive agents and half doses were used to avoid profound symptomatic hypo/hypertension in these individuals. Changes in muscle sympathetic nerve activity and heart rate were measured in response to the increase and decrease in blood pressure. Pharmacological sBRS was calculated by the BRSA method described above (see Section 5.4.1.2.1), the method for calculating pharmacological cBRS is described below (see Section 5.4.1.2.3.2).

5.4.1.2.3 Measurement of cardiac baroreflex sensitivity

5.4.1.2.3.1 Spontaneous cardiac baroreflex sensitivity

Spontaneous cardiac baroreflex sensitivity was assessed using the sequence technique (Bertinieri, Di Rienzo et al. 1988, Parati, Di Rienzo et al. 1988). The sequence technique is based on the identification of sequences of consecutive beats in which progressive increases in SBP are followed with a one-beat delay by a progressive lengthening in RR interval, or vice versa (Di Rienzo, Parati et al. 2001). It is possible to calculate cBRS over a range of cardiac cycle lags between the paired SBP and RRI, for logistical reasons I have calculated spontaneous cBRS for a 1 beat lag only, in keeping with the predominant method in the literature (Di Rienzo, Parati et al. 2001). At normal resting heart rate of 60-80 bpm a 1 beat lag represents the average baroreflex latency of 1.2 seconds, at faster heart rates a longer lag should be used (Hart, Joyner et al. 2010). Sequences of three or more successive heart beats in which there are simultaneous, concordant increases or decreases in SBP and RR interval (RRI) were identified. A linear regression was applied to the relationship between SBP and RRI for each of the sequences and an average regression slope was calculated for the sequences detected during each recording period. This slope represents the overall spontaneous cardiac baroreflex sensitivity. Data were also analysed separately to quantify the spontaneous cBRS to both

rising and falling SBP. Values of cBRS were accepted when the number of sequences was ≥3 for both rising and falling sequences (Taylor, Witter et al. 2015).

When using 5-10-minute recordings we frequently found that our datasets contained <3 concordant sequences, and therefore we were unable to quantify spontaneous cBRS for that recording. In order to address this issue, we also calculated the baroreceptor effectiveness index (BEI) for each dataset. In normal individuals, concordant SBP/RRI sequences occur at a rate of around 80/24 hours (Di Rienzo, Parati et al. 2001), this means that it is not surprising to only obtain 1-2 concordant sequences (ramps) in a short recording. Even in healthy volunteers, SBP ramps also occur in the absence of concordant RRI ramps, indicating variable coupling of heart rate in response to changes in BP (Di Rienzo, Parati et al. 2001). The BEI quantifies this phenomenon and is defined by the ratio between the total number of concordant SBP/RRI ramps and the total number of SBP ramps (Di Rienzo, Parati et al. 2001). For practical reasons the BEI has been calculated using ramps with a 1 beat lag only.

5.4.1.2.3.2 Pharmacological cardiac baroreflex sensitivity

Whilst the sequence technique is commonly used to assess spontaneous cBRS, published data more frequently uses a 'binning' method (similar to sBRS analyses) to quantify cBRS in the context of the modified Oxford protocol (Minson, Halliwill et al. 2000, Halliwill and Minson 2002, Dutoit, Hart et al. 2010, Barnes, Matzek et al. 2012); this may in part reflect the small number of sequences available for analysis. Data for RRI collected during the modified Oxford protocol (including 2-minute recovery period) were pooled into 2 mmHg SBP bins (with 1 beat lag). The mean RRI and SBP for each bin was calculated. The slope of the linear relationship between mean RRI and mean SBP quantified the pharmacological cBRS (Charkoudian, Martin et al. 2004, Barnes, Matzek et al. 2012).

5.4.1.2.3.3 Correlations versus blood pressure, sympathetic nerve activity, and target organ damage

Changes in BRS have been correlated against both baseline measures of and changes in office SBP and measures of cardiac volumetrics and function, and aortic distensibility, and also versus muscle sympathetic nerve (MSNA) and heart rate variability (HRV) parameters, as assessed before, and six months after, RDN. The methods for these measures are described in Sections 4.3.4, 4.3.5 and 4.3.6, and the results for the correlations are presented in full in Appendices 2 and 3, positive and significant negative data are presented in detail in the relevant sections of this chapter. Only correlation analyses with a minimum of 5 data pairs are presented.

5.4.1.3 Results

5.4.1.3.1 Spontaneous sympathetic baroreceptor sensitivity

Spontaneous sympathetic BRS data were available for 13/18 patients (14 patients with MSNA, error with DBP recording in 1 participant therefore unable to calculate sBRS). Baseline office SBP correlated with baseline falling spontaneous sBRST and falling

spontaneous sBRSA (R=-0.63, p=0.02; R=-0.58, p=0.04, respectively, see Figure 5-26). Left ventricular mass (LVM) index was also negatively correlated with overall spontaneous sBRST at baseline (R=-0.65, p=0.02). Baseline MSNA incidence did not correlate with any of the baseline spontaneous sBRS parameters. Spontaneous sBRST and sBRSA were strongly correlated at baseline (see Figure 5-27).

In the 10 participants with spontaneous sBRS available at baseline and six months post-RDN, there were no changes in any of the spontaneous sBRS variables at this primary endpoint as assessed by paired Student's t-test (see Table 5-17). Furthermore, there were no significant changes in any of the spontaneous sBRS parameters over the full course of the study as assessed by repeated-measures ANOVA (n=13, see Table 5-18). There was no correlation between the change in office SBP and the change in any of the spontaneous sBRS parameters at 6 months post-RDN. Likewise, there were also no correlations between the changes in either MSNA incidence, HRV parameters, indexed LVM or aortic distensibility, and the change in any of the spontaneous sBRS parameters at 6 months.

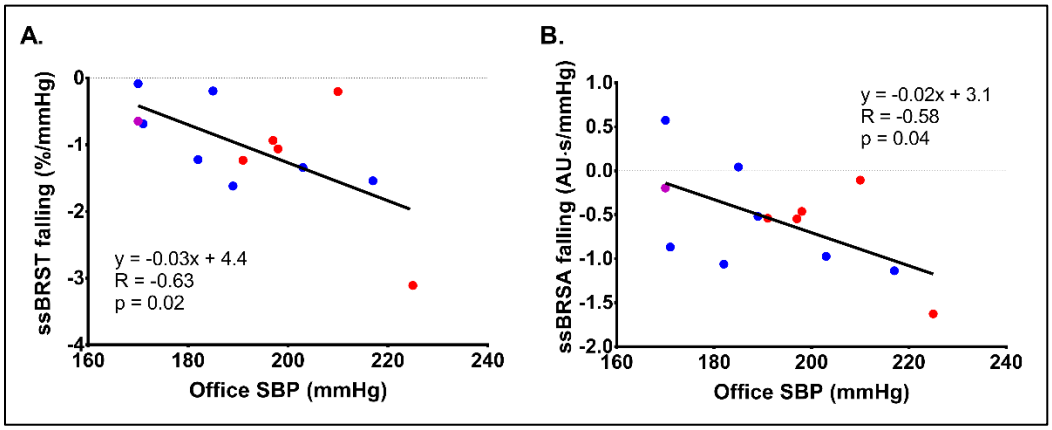


Figure 5-26. Correlation between baseline office systolic blood pressure (SBP) and baseline spontaneous sympathetic baroreflex sensitivity for falling pressures (ssBRS). Relationship between office SBP and ssBRS assessed by A. the threshold method, and B. the area method. Significance taken as $p<0.05$. Male participants' data are depicted in blue, pre-menopausal women in red and post-menopausal women in purple.

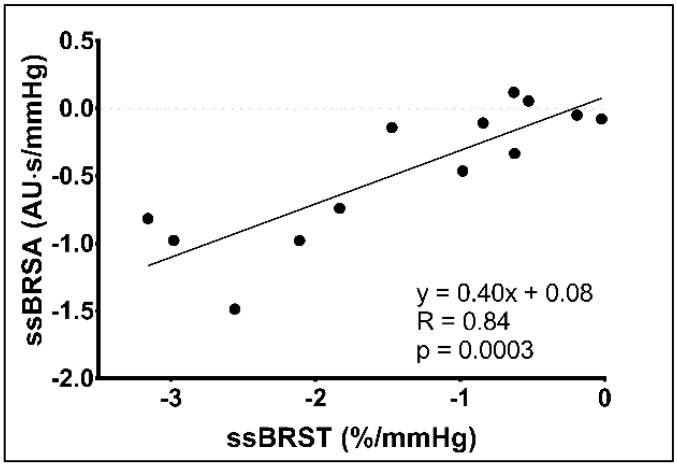


Figure 5-27. Strong correlation between baseline spontaneous sympathetic BRS assessed by the threshold versus area method.
ssBRST; spontaneous sympathetic baroreflex sensitivity – threshold method, ssBRSA; spontaneous sympathetic baroreflex sensitivity – area method. Significance taken as $p<0.05$.

Parameter	Time post RDN (months)		P
	0	6	
BRST overall (%/mmHg)	-1.55 ± 0.37	-1.20 ± 0.45	0.59
BRST rising (%/mmHg)	-1.11 ± 0.41	-1.29 ± 0.29	0.73
BRST falling (%/mmHg)	-1.07 ± 0.29	-0.87 ± 0.53	0.75
BRSA overall (AU•s/mmHg)	-0.55 ± 0.16	-0.73 ± 0.24	0.49
BRSA rising (AU•s/mmHg)	-0.66 ± 0.26	-0.90 ± 0.22	0.47
BRSA falling (AU•s/mmHg)	-0.50 ± 0.20	-0.70 ± 0.26	0.55

Table 5-17. No change in spontaneous sympathetic baroreflex as assessed by the threshold (BRST) or area (BRSA) methods assessed at baseline and 6 months following renal denervation (RDN).
Data shown for 10 participants with ssBRS available at baseline and 6 months post-RDN. Data reported for overall BRS, and then BRS to either rising or falling diastolic blood pressure. P value is for paired Student's t-test and data are reported as mean ± SEM.

Parameter	Time post RDN (months)					P
	0	1	3	6	12	
BRST overall (%/mmHg)	-1.38 ± 0.29	-1.74 ± 0.35	-1.99 ± 0.26	-1.42 ± 0.39	-1.16 ± 0.47	0.53
BRST rising (%/mmHg)	-1.30 ± 0.41	-1.64 ± 0.40	-1.82 ± 0.35	-1.38 ± 0.29	-1.60 ± 0.43	0.90
BRST falling (%/mmHg)	-1.07 ± 0.22	-0.72 ± 0.56	-0.99 ± 0.45	-1.10 ± 0.47	-0.82 ± 0.68	0.97

BRSA overall (AU•s/mmHg)	-0.46 ± 0.14	-0.78 ± 0.18	-1.00 ± 0.13	-0.76 ± 0.19	-0.70 ± 0.24	0.33
BRSA rising (AU•s/mmHg)	-0.67 ± 0.21	-0.78 ± 0.20	-1.01 ± 0.23	-0.92 ± 0.19	-0.96 ± 0.27	0.80
BRSA falling (AU•s/mmHg)	-0.57 ± 0.16	-0.86 ± 0.29	-0.77 ± 0.27	-0.84 ± 0.23	-0.68 ± 0.34	0.89

Table 5-18. No change in spontaneous sympathetic baroreflex as assessed by the threshold (BRST) or area (BRSA) methods following renal denervation (RDN).
Data shown for 13 participants for overall BRS, and then BRS to either rising or falling diastolic blood pressure. P value is for repeated measures on-way ANOVA, there were no significant differences between groups on Bonferroni comparison. Data shown is mean ± SEM.

In summary, patients with higher baseline oSBP and iLVM had greater baseline spontaneous sBRS, but there was no change in spontaneous sBRS following RDN.

5.4.1.3.2 Pharmacological sympathetic baroreflex sensitivity

Five of the ten participants who underwent the Modified Oxford protocol had adequate quality MSNA and DBP data at baseline for analysis of pharmacological sBRSA. Each of these participants had follow-up data available at either 6 or 12 months post-RDN, and therefore data have been analysed from baseline versus a composite endpoint of 6-12 months follow-up.

At baseline, there were no significant correlations between overall, rising or falling spontaneous sBRSA and overall, rising or falling pharmacological sBRSA (all $p>0.05$). Baseline office SBP was negatively correlated with baseline overall pharmacological sBRSA (n=5, R=-0.90, p=0.04, see Figure 5-28) and baseline pharmacological sBRSA (n=5, R=-0.91, p=0.03) for falling DBP. Baseline MSNA incidence was positively correlated against baseline overall pharmacological sBRSA (n=5, R=0.90, p=0.04, see Figure 5-28) and baseline pharmacological sBRSA for falling DBP (n=5, R=0.90, p=0.04). Higher baseline office SBP was associated with greater (more negative) BRS, whereas higher baseline MSNA was associated with lower BRS (see Figure 5-28). There were no significant correlations between baseline overall pharmacological sBRSA and baseline heart rate variability parameters, baseline aortic distensibility or any of the baseline CMR volumetric or strain parameters (including LVM, all $p>0.05$).

There was no significant change in pharmacological sBRSA in response to overall changes in DBP or in response to rising or falling DBP specifically, following RDN (see Table 5-19). There was no significant correlation between the change in overall pharmacological sBRSA at 6-12 months post-RDN and the change oSBP at six months after the procedure (n=5, R=0.48, p=0.42). Likewise, there was no significant correlation between the change in overall pharmacological sBRSA and the change in MSNA, the changes in HRV parameters, the change in aortic distensibility or the changes in any of the CMR parameters following RDN.

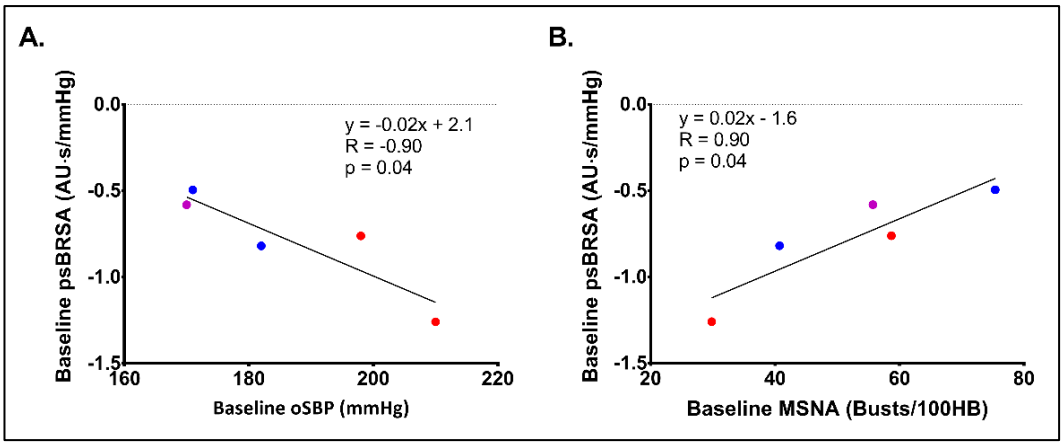


Figure 5-28. Correlations between baseline pharmacological sympathetic baroreflex sensitivity (psBRSA) versus A. baseline office systolic blood pressure (oSBP) and B. baseline muscle sympathetic nerve activity (MSNA).
Baroreflex sensitivity assessed in response to the Modified Oxford protocol with assessment of MSNA area. Data are for a Pearson's correlation. Male participants' data are depicted in blue, pre-menopausal women in red and post-menopausal women in purple.

Parameter	Time post RDN (months)		P
	0	6-12	
psBRSA overall (AU•s/mmHg)	-0.78 ± 0.13	-0.87 ± 0.15	0.30
psBRSA rising (AU•s/mmHg)	-0.81 ± 0.14	-0.86 ± 0.20	0.83
psBRSA falling (AU•s/mmHg)	-0.52 ± 0.11	-0.52 ± 0.13	0.98

Table 5-19. No significant change in baroreflex sensitivity as assessed using the modified Oxford protocol.
There were no significant changes in pharmacologically tested, sympathetic baroreflex sensitivity assessed by area method (psBRSA), either for all data, or for data in response to rising or falling diastolic blood pressure, with follow-up data assessed at 6-12 months after renal denervation (RDN). P value for paired Students t-test, n=5.

In summary, higher baseline office SBP was associated with greater (more negative) BRS, whereas higher baseline MSNA was associated with lower pharmacological sBRS at baseline, but there was no change in pharmacological sBRS following RDN.

5.4.1.3.3 Spontaneous cardiac baroreflex sensitivity

Baseline spontaneous cardiac baroreflex sensitivity was assessed in 16/18 participants, however, when using the sequence method for analysis for overall, rising and falling changes in SBP only 13, 13, and 11 participants, respectively, had ≥3 concordant sequences from which to calculate an average regression line representing spontaneous cBRS. In the analyses below only results for spontaneous cBRS based on ≥3 sequences have been included. An example showing the calculation of spontaneous cBRS using the sequence method is shown in Figure 5-29. Baroreflex effectiveness index could be calculated in all 16 patients with baseline spontaneous cBRS data.

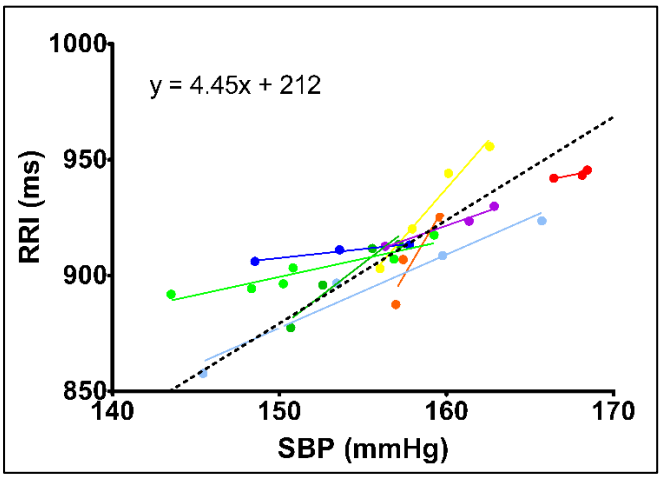


Figure 5-29. Example of calculation of spontaneous cardiac baroreflex sensitivity (scBRS) using the sequence method.
Concordant sequences are shown as coloured data points with linear regression. The scBRS is the mean of these regression lines and is shown as a black dotted line, giving a scBRS of 4.45 ms/mmHg in this example. RRI; R wave to R wave interval, SBP; systolic blood pressure.

At baseline, there were no significant correlations between office SBP and overall, rising and falling spontaneous cBRS and BEI. The correlation between baseline oSBP and baseline spontaneous cBRS for falling SBP approached significance (n=11, R=-0.60, p=0.05, see Figure 5-30), with participants with higher SBP having lower (impaired) spontaneous cBRS. When looking at baseline spontaneous cBRS parameters correlated against baseline HRV parameters, there was a significant correlation between baseline overall spontaneous cBRS and baseline low frequency (LF) spectral components (n=13, R=0.87, p=<0.0001, see Figure 5-30), with patients with higher oSBP having higher LF spectral measures, which may be consistent with higher cardiac sympathetic nerve activity (SNA). Baseline BEI for all sequences was inversely correlated against baseline normalised high frequency (nHF) power (n=16, R=-0.50, p=0.049, see Figure 5-30), with individuals with higher BEI (greater cBRS concordance) having lower nHF power, and thus lower vagal input to HRV. There were no correlations between baseline MSNA incidence and any of the baseline spontaneous cBRS or BEI measures (all p>0.05).

There were no significant correlations between spontaneous cBRS for all sequences and any of the cardiac volumetric or strain parameters. Baseline spontaneous cBRS for falling SBP sequences correlated against baseline left ventricular mass (n=11, R=-0.66, p=0.03,

see Figure 5-30), approaching significance for indexed LVM (n=11, -0.58, p=0.06), with patients with higher (better) spontaneous cBRS having lower LVM. There were no correlations between baseline aortic distensibility and any of the measures of baseline spontaneous cBRS or BEI (all p>0.05).

Follow-up spontaneous cardiac BRS data were available at the primary 6-month endpoint in 16 participants and was used to calculate a BEI, however, only 9 participants had spontaneous cBRS data calculated using ≥3 sequences which could be used for outcome analyses. There was a significant increase in spontaneous cBRS for the response to combined rising and falling SBP from baseline to 6 months (n=9, 7.0 ± 1.7 ms/mmHg vs 9.9 ± 2.1 ms/mmHg, respectively, p=0.048, see Table 5-20).

When data were assessed over the full study time course (using repeated-measures ANOVA with data carried forward), there were also no significant changes in any of the spontaneous cBRS parameters following RDN (see Table 5-21). However, there were possible trends towards an increase in spontaneous cBRS for falling SBP sequences (p=0.09) and for a change in overall BEI (p=0.08), with the latter peaking at baseline and 6 months post-RDN.

There were no significant correlations between the change in either office SBP or MSNA at 6 months after RDN and the changes in any of the spontaneous cBRS parameters following RDN (all p>0.05).

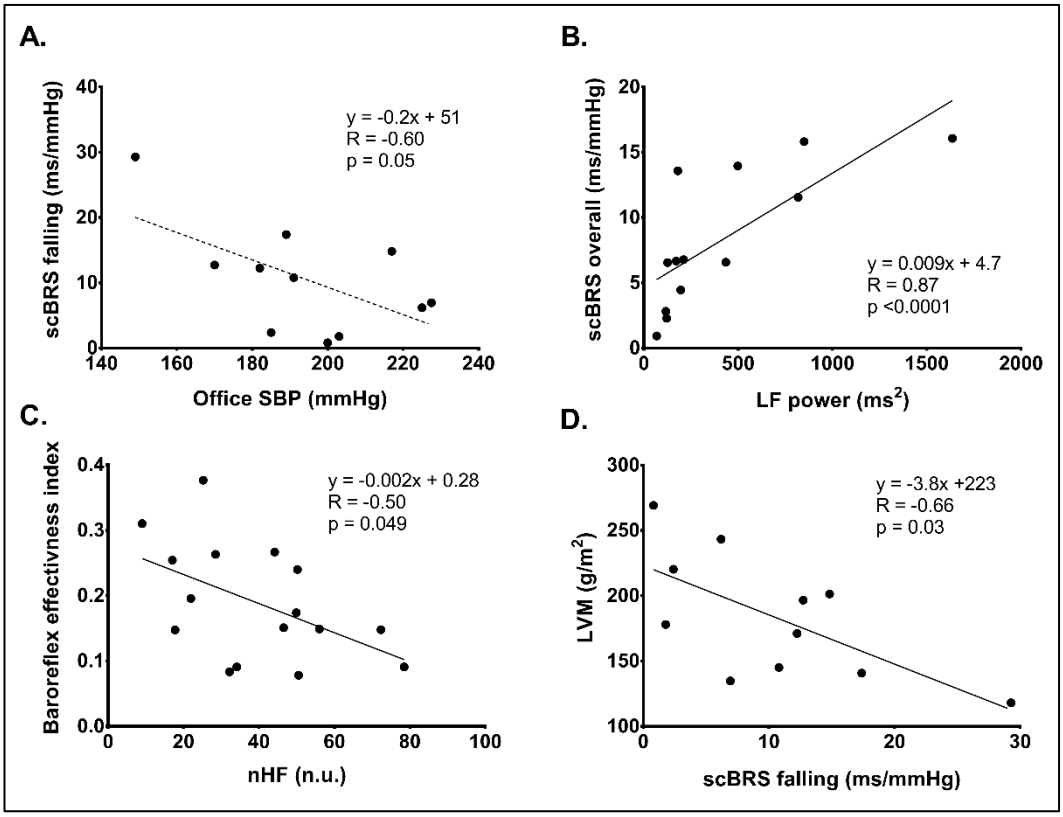


Figure 5-30. Correlations versus baseline spontaneous cardiac baroreflex sensitivity (scBRS).

A. Office systolic blood pressure (SBP) versus scBRS for falling SBP, B. low frequency (LF) spectral heart rate variability (HRV) versus overall scBRS, C. normalised high frequency (nHF) spectral HRV versus baroreflex effectiveness index, and D. scBRS for falling SBP versus left ventricular mass (LVM). Patients with higher baseline SBP trended towards

lower (impaired) scBRS, those with high LF power (indicating increased sympathetic nerve activity) had higher (increased) spontaneous cardiac baroreflex gain whereas individuals with high nHF spectral components (indicating increased vagal activity) had poor concordance between changes in SBP and RRI. Poor scBRS was associated with increased LVM.

At six months post-RDN, there were significant correlations between the change in overall spontaneous cBRS and the changes in the HRV parameters nHF spectral power (n=7, R=0.76, p=0.49) and LF/HF ratio (n=7, R=-0.86, p=0.02, see Figure 5-31). There were also correlations between the change in spontaneous cBRS for rising SBP and the changes in nHF and nLF power (n=7, R=0.86, p=0.01, and n=7, R=-0.81. p=0.03), and the change in LF/HF ratio (n=7, R-0.79, p=0.048). Furthermore, at six months after the procedure, there were significant correlations between the change in overall BEI and the changes in nHF power (n=13, R=-0.59, p=0.04), LF power (n=13, R=0.71, p=0.006) and LF/HF ratio (n=13, R=0.70, p=0.008, see Figure 5-31). There were also correlations between the change in BEI for falling SBP and the changes in nHF power (n=13, R=-0.68, p=0.01), nLF power (n=13, R=0.57, p-0.04), LF power (n=13, R=0.60, p=0.03) and LF/HF ratio (n=13, R=0.87, p=0.0001).

Parameter		**Time post RDN (months)**		
	N	0	6	P
scBRS overall (ms/mmHg)	9	7.0 ± 1.7	9.9 ± 2.1	0.048
scBRS rising (ms/mmHg)	8	6.7 ± 1.4	8.9 ± 1.7	0.15
scBRS falling (ms/mmHg)	6	9.7 ± 2.5	14.4 ± 3.6	0.15
BEI overall	16	0.19 ± 0.02	0.17 ± 0.03	0.67
BEI rising	16	0.20 ± 0.03	0.16 ± 0.03	0.34
BEI falling	16	0.19 ± 0.03	0.17 ± 0.04	0.82

Table 5-20. Spontaneous cardiac baroreflex sensitivity (scBRS) parameters at baseline versus 6 months after renal denervation (RDN).
BEI; baroreflex effectiveness index. P values for paired Student's t-test.

Parameter	**Time post RDN (months)**					**P**
	0	1	3	6	12	
scBRS overall (ms/mmHg)	7.7 ± 1.4	10.0 ± 3.2	10.4 ± 3.0	10.0 ± 1.7	10.1 ± 3.9	0.41
scBRS rising (ms/mmHg)	5.7 ± 1.1	8.6 ± 3.1	8.3 ± 3.1	7.3 ± 1.3	7.2 ± 1.8	0.82
scBRS falling (ms/mmHg)	8.9 ± 2.1	8.2 ± 1.8	7.8 ± 1.7	12.9 ± 2.9	18.1 ± 7.8	0.09

BEI overall	0.19 ± 0.02	0.13 ± 0.03	0.14 ± 0.03	0.17 ± 0.03	0.13 ± 0.02	0.08
BEI rising	0.20 ± 0.03	0.13 ± 0.03	0.14 ± 0.04	0.16 ± 0.03	0.15 ± 0.03	0.24
BEI falling	0.19 ± 0.03	0.13 ± 0.03	0.14 ± 0.04	0.17 ± 0.04	0.12 ± 0.03	0.20

Table 5-21. Change in spontaneous cardiac baroreflex sensitivity (scBRS) following renal denervation (RDN).
For scBRS, qualifying data required ≥3 concordant sequences, giving n= 12, n=12 and n=8 for overall, rising and falling scBRS, respectively. Analysis of baroreflex effectiveness index (BEI) does not require a minimum number of concordant sequences and was therefore quantified in all 16 participants with available scBRS data. Data were assessed by repeated-measures ANOVA with data carried forward from the previous study visit to fill and data gaps.

Finally, there were no significant correlations between the changes in the spontaneous cBRS or BEI parameters and changes in any of the cardiac volumetric or strain parameters, or aortic distensibility, at 6 months after renal denervation (all p>0.05).

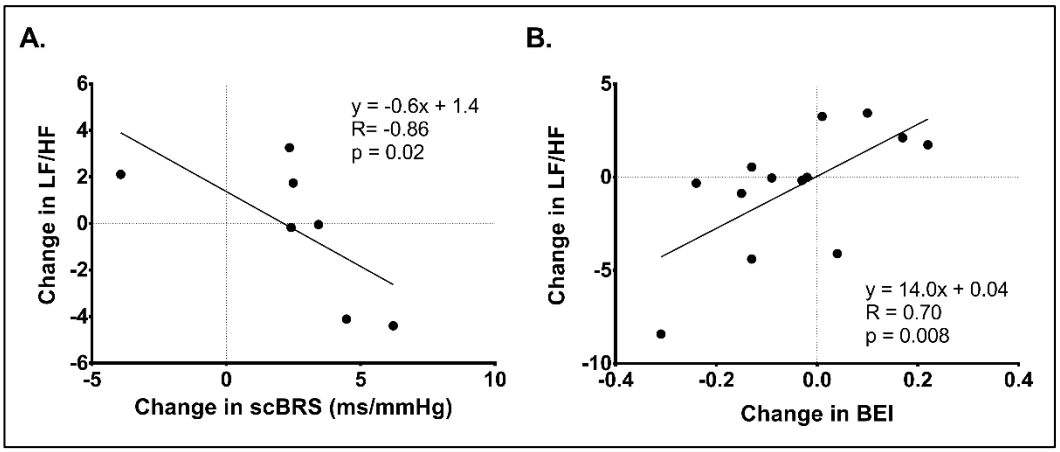


Figure 5-31. Changes in measures of spontaneous cardiac baroreflex sensitivity (A.) and effectiveness index (B.) correlate with change in sympathovagal balance at 6 months after renal denervation,
A fall in LF/HF, a measure of cardiac sympathovagal balance was associated with an improvement in cardiac baroreflex sensitivity, but a reduction in the transduction of changes in BP into changes in heart rate. scBRS; spontaneous sympathetic baroreflex sensitivity, BEI; baroreflex effectiveness index, LF/HF; low frequency/high frequency heart rate variability spectral power ratio.

In summary, there was a trend for participants with higher baseline SBP to have lower (impaired) spontaneous cBRS at baseline, and individuals with higher (better) spontaneous cBRS had a lower LVM. There was a significant increase in spontaneous cBRS following RDN, which was independent of any changes in oSBP or MSNA. To summarise the relationship between changes in spontaneous cBRS and changes in the HRV parameters post-RDN; as vagal activity increases, and/or cardiac sympathetic nerve

activity decreases (decreased LF/HF ratio), spontaneous cardiac BRS improved, but BEI decreased (see Figure 5-31).

5.4.1.3.4 Pharmacological cardiac baroreflex sensitivity

Pharmacological cardiac baroreflex sensitivity was calculated in response to the modified Oxford protocol in 10/18 participants at baseline. Mean overall pharmacological sBRS at baseline was 3.0 ± 0.7 ms/mmHg. Pharmacological sBRS data were then available in 7, 6, 8 and 5 participants at 1, 3, 6 and 12 months respectively.

At baseline, pharmacological cBRS was strongly correlated against spontaneous cBRS. There were no correlations between baseline pharmacological cBRS and baseline office SBP (n=10, R=-0.25, p=0.49) and baseline MSNA incidence (n=7, R=-0.29, p=0.56). Baseline pharmacological cBRS correlated with baseline LF HRV power (n=10, R=0.82, p=0.006), which could indicate that those with higher pharmacological cBRS have higher cardiac sympathetic nerve activity (there were no correlations with any of the other spectral components).

Pharmacological cBRS at baseline did not correlate significantly against any of the baseline cardiac volumetric or strain MRI data or baseline aortic distensibility.

When considering the primary study outcome visit, there was no significant change in pharmacological cBRS between baseline and 6 months post-RDN amongst the eight participants with data available at both timepoints (3.2 ± 0.8 ms/mmHg vs 3.8 ± 0.7 ms/mmHg, p=0.15 (Wilcoxon matched-pairs signed rank test)). When data were analysed across all study time-points there was also no significant change in pharmacological sBRS following RDN (see Table 5-22).

Parameter	**Time post RDN (months)**					**P**
	0	1	3	6	12	
pcBRS overall (ms/mmHg)	3.0 ± 0.7	3.8 ± 1.1	4.7 ± 1.2	3.4 ± 0.6	3.6 ± 0.8	0.80

Table 5-22. No change in pharmacological cardiac baroreflex sensitivity (pcBRS) over 12 months following renal denervation (RDN).
Data for all 10 participants with baseline psBRS data, analysed by Friedman test, with data carried forward from previous study visit to cover any missing results (data available in 10, 7, 6, 8 and 5 participants at 0, 1, 3, 6 and 12 months, respectively). No significant differences on between group analysis.

There were no significant correlations between the change in pharmacological sBRS and the changes in spontaneous sBRS (both BRST and BRSA; n=5, R=-0.10, p=0.95) at 6 months post-RDN. There were also no significant correlations between the change in pharmacological cBRS and the changes in office SBP (n=8, R=-0.12, p=0.79), MSNA (n=4, R=0.80, p=0.33) and any of the HRV parameters (all n=8, p>0.05) at six months after the procedure.

When considering target organ damage, there were no correlations between the change in pharmacological cBRS and the changes in any of the volumetric or strain CMR parameters or versus the change in aortic distensibility at months following RDN (all $p>0.05$).

In summary, there was no significant changes in pharmacological cBRS following RDN.

5.4.1.4 Discussion

5.4.1.4.1 Effect of renal denervation on sympathetic baroreflex sensitivity

These outcome data for each of the measures of spontaneous and pharmacological sBRS consistently demonstrate no change in sympathetic baroreflex sensitivity following RDN. These results are in conflict with the conclusions drawn in Sections 5.3.4.2 and 5.3.5, since there was a reduction (or at least a trend towards a reduction at 6 months) in office SBP following RDN, but no change in MSNA, which would suggest that there had been a resetting in baroreflex gain (Guyenet 2006, Hart, Joyner et al. 2010). The outcomes in the present study differ from existing published data. Our group has previously reported an improvement in spontaneous sBRS following RDN, which was independent of a change in BP, in a cohort of 8 patients (4 of whom are in the present study) (Hart, McBryde et al. 2013). This translational study was further supported by improvements in pharmacological sBRS assessed in 7 spontaneously hypertensive rats which had received surgical RDN (Hart, McBryde et al. 2013). Grassi et al. reported reductions in both MSNA and spontaneous sBRS at 3 and 6 months following RDN in 15 patients with resistant hypertension treated with RDN, furthermore, the reduction in MSNA was associated with an improvement in spontaneous sBRS, although the changes in both of these variables were independent of any change in BP (Grassi, Seravalle et al. 2015). The number of participants studied in any of these studies remains small and the findings warrant further investigation.

At baseline, office SBP was negatively correlated with spontaneous sBRS in response to falling DBP (see Figure 5-26), and when considering the baseline pharmacological sBRS data, higher baseline office SBP was also associated with greater (more negative) BRS, whereas higher baseline MSNA was associated with lower BRS, particularly in response to falling BP (see Figure 5-28). At a simplistic level, hypertension is associated with impaired BRS (Yamada, Miyajima et al. 1988, Hesse, Charkoudian et al. 2007, Grassi, Seravalle et al. 2014), however, it is important to differentiate between sympathetic input to cardiac BRS and sympathetic vasomotor baroreflex sensitivity. The majority of papers in this field refer to cardiac BRS, which is more readily measured on a population basis, with impaired cardiac baroreflex gain established as a poor prognostic marker for cardiovascular disease (Ormezzano, Cracowski et al. 2008). In young adults, cardiac BRS is not correlated with sympathetic BRS (Dutoit, Hart et al. 2010), and the picture is complicated by hysteresis in the sympathetic baroreflex response to rising versus falling DBP (Hart, Wallin et al. 2011). The sensitivity of the sympathetic baroreflex depends on the level of resting sympathetic tone: individuals with high resting SNA (usually older men and women and/or those with hypertension) respond better to rising BP changes, whereas those with low MSNA (younger men and women) respond better to falling BP

changes (Hart, Wallin et al. 2011). This picture is further complicated in young females in whom there is poor correlation between BP and MSNA (Hart, Charkoudian et al. 2009, Hart, Charkoudian et al. 2011). Higher baseline SBP was associated with increased sBRS in our study, and since raised BP in usually associated with raised MSNA, this may seem counterintuitive. However, in this cohort, 5/13 patients with baseline spontaneous sBRS and 2/5 patients with baseline pharmacological sBRS were premenopausal females. As discussed in Section 5.3.4.2, there was an inverse relationship between baseline SBP and baseline MSNA amongst our participants which we hypothesise relates to the significant proportional of younger females, some of whom had very high baseline SBP despite low/normal range MSNA, and once again the dissociation between MSNA and BP in these participants is likely to impact the sBRS findings.

5.4.1.4.2 Effect of renal denervation on cardiac baroreflex sensitivity

There was a significant increase in overall spontaneous cardiac BRS (in response to combined rising and falling SBP) from baseline to 6 months after denervation (see Table 5-20). In contrast, there was no significant change in pharmacological cardiac BRS this primary study endpoint. When data were assessed over the full study time course (using repeated-measures ANOVA with data carried forward), there were also no significant changes in any of the spontaneous cBRS or pharmacological cBRS parameters following RDN. However, there were possible trends towards an increase in spontaneous cBRS for falling SBP sequences (p=0.09) and for a change in overall BEI (p=0.08), with the latter peaking at baseline and 6 months post-RDN. These data indicate an improvement in spontaneous cBRS following RDN, but not consistently so.

There is little published data on the effect of RDN on cardiac BRS. Translational data from our group previously reported improvements in cBRS in both rats and humans following RDN (human data 4/8 patients also presented in the current study) (Hart, McBryde et al. 2013). In a rat model of cisplatin-induced renal failure, bilateral renal denervation was shown to restore impaired cBRS in response to a volume load (Khan, Sattar et al. 2014), likewise, there was an improvement in cBRS following RDN in a rat Goldblatt hypertension model (Lincevicius, Shimoura et al. 2017). Zuern et. al identified cBRS as a potential predictor of response to RDN, but unfortunately did not publish follow-up data on the cBRS findings post-denervation (Zuern, Eick et al. 2013). The effect of RDN on cBRS therefore remains to be established in larger human studies, but these animal and pilot data support other findings of an improvement in spontaneous cardiac BRS following RDN. The mechanism for this relationship remains to be established, particularly as to whether it is purely due to a reduction in BP or whether there is modulation of the cardiac baroreflex through changes in central and/or cardiac sympathetic tone.

At six months post-RDN, amongst other correlations, there were significant associations between the changes in overall spontaneous cBRS and spontaneous cBRS for rising SBP, and the changes in LF/HF ratio, a marker of sympathovagal balance (see Figure 5-31). There were also significant correlations between the changes in overall BEI and BEI for falling SBP, and the change in LF/HF ratio (see Figure 5-31). These data would indicate that as vagal activity increases, and/or cardiac sympathetic nerve activity decreases (decreased LF/HF ratio), spontaneous cardiac BRS improved. An improvement in cBRS

following a reduction in SNA after RDN would be consistent with the preliminary data from our group and from animal studies (Hart, McBryde et al. 2013, Khan, Sattar et al. 2014, Lincevicius, Shimoura et al. 2017), but one might have expected an increase in BEI post-RDN in association with increased LF/HF balance if BEI were a marker of cardiac baroreflex gain. cBRS and BEI are, in fact, quantifying two different aspects of the relationship between RRI and BP: cardiac baroreflex sensitivity quantifies the magnitude of the baroreflex responses (the mean gradient of the regression line), whereas BEI measures how much of the BP stimuli are being transduced into a response (i.e. the percentage of change in BP that is effectively transmitted through the neural pathways into a reflex HR response) (Di Rienzo, Parati et al. 2001, Silva and Katayama 2017). The use of the sequence method to assess cBRS, particularly when applied to shorter duration recordings, is intrinsically limited by the number of concordant SBP and RRI ramps and is therefore impacted by the efficacy of cardiac baroreflex transduction. In healthy humans, the mean BEI was 21% with an average of 363 SBP ramps in 24 hours (Di Rienzo, Parati et al. 2001). It is therefore not surprising that over a 5 min recording we struggled to identify ≥3 concordant sequences to enable quantification of the mean gradient, and thus spontaneous cBRS. Based on the data from Di Rienzo et al. it might be expected to measure, on average only 1.3 SBP ramps in a 5-minute recording (Di Rienzo, Parati et al. 2001). BEI has the benefit that it takes into account all SBP ramps in a recording, rather than only concordant SBP/RRI ramps, and therefore could be quantified is a greater proportion of our participants (overall spontaneous cBRS reported for 13/18 participants, BEI reported for 16/18 participants). BEI may therefore reflect a more readily available measure of the reflex relationship between SBP and RRI, but it must not be forgotten that BEI assesses a different aspect of this relationship to spontaneous cBRS and further research is required before the prognostic data established for cBRS can be extrapolated to the BEI.

5.4.1.4.3 Baroreflex sensitivity and target organ damage following renal denervation

At baseline, individuals with reduced overall spontaneous sBRST had a lower LVM index. One might have expected impaired spontaneous sBRS to be associated with increased LVM and left ventricular hypertrophy (Grassi, Seravalle et al. 2009), but as with the correlation between baseline oSBP and baseline spontaneous sBRS described above, this counterintuitive relationship may reflect the proportion of premenopausal women in this small cohort and should be further investigated in a larger population.

There was, however, a significant correlation between baseline spontaneous cBRS for falling SBP sequences versus baseline LVM (approaching significance for indexed LVM), with patients with higher (better) spontaneous cBRS having lower LVM, which is more consistent with previous data reporting an inverse relationship between cBRS and LVM (Milan, Caserta et al. 2007, Grassi, Seravalle et al. 2009, Song, Kim et al. 2012).

Despite the relationships seen at baseline, there were no correlations between changes in any of the CMR parameters and changes in any of the cardiac or sympathetic BRS at six months after renal denervation. Given that there was a significant reduction in LVM over the course of the study (see Table 5-6), and that impaired BRS, particularly cBRS, has been previously associated with hypertensive heart disease and is an independent marker of the risk of mortality and major adverse cardiovascular events in hypertensive

patients (Ormezzano, Cracowski et al. 2008, Grassi, Seravalle et al. 2009), one might have predicted an improvement in cBRS following RDN. Cardiac BRS did indeed improve in our cohort at 6 months post-RDN, but this change did not correlate with the reduction in LVM. This may reflect the small size of the cohort, insufficient duration of follow-up, or a more complex mechanistic relationship between BRS and hypertensive heart disease.

5.4.2 Sympathetic neuro-vascular transduction

5.4.2.1 Introduction

Thus far, we have focussed on the relationship between RDN and muscle sympathetic nerve activity, with measurement of the electrical activity from the sympathetic nerves supplying the vasoactive blood vessels in the leg. In order for these action potentials to be converted into changes in vascular tone there must be transduction across the neuromuscular junction. Sympathetic nerve activity triggers the release of noradrenaline from the postganglionic nerve terminals at the neuromuscular junction, which then primarily acts upon α1-adrenergic receptors on the vascular smooth muscle cells resulting in vasoconstriction (Guyton and Hall 1996). There are lots of factors which can impact sympathetic neuro-haemodynamic transduction, including the release of vasoactive co-transmitters which can act both pre- and post-synaptically, the relative concentration of α1 and α2 (vasoconstrictive) and β2 (vasodilatory) adrenergic receptors on the post-synaptic smooth muscle cells, the relative pre-synaptic concentrations of α2 (inhibit noradrenaline release) and β2 (facilitate noradrenaline release) adrenergic receptors, and the presence of other locally acting vasoactive substances such as nitric oxide (Brodde 1990, Guyton and Hall 1996, Kneale, Chowienczyk et al. 2000, Pablo Huidobro-Toro and Veronica Donoso 2004, Guyenet 2006, Burnstock 2008, Briant, Burchell et al. 2016). The efficacy of sympathetic neurovascular transduction must also be evaluated in order to fully appreciate the relationship between arterial pressure, baroreflex sensitivity, muscle sympathetic nerve activity and vascular tone.

5.4.2.2 Method

We aimed to assess the transduction of sympathetic nerve activity into vascular tone by quantifying the relationship between MSNA burst area and change in diastolic blood pressure (Briant, Burchell et al. 2016). As with the quantification of sympathetic BRS, DBP was used as a proxy for the vascular response because: (i) it is easily and reliably measured, (ii) it is a target variable regulated by SNA, and (iii) studies show that DBP reflects sympathetic vasomotor tone (Barnes, Hart et al. 2014, Briant, Burchell et al. 2016). Resting beat-to-beat BP, three-lead ECG and MSNA were measured continuously over a 5-10-minute period as described in Section 4.3.

Sympathovascular transduction was quantified as previously described (Briant, Burchell et al. 2016). For each recorded DBP (each cardiac cycle), the summed MSNA burst area was measured at a fixed cardiac cycle lag of 8–6 cardiac cycles (see Figure 5-32). A fixed lag of 8-6 cardiac cycles was used since this has previously been shown to give the peak

transduction slope for this relationship (Briant, Burchell et al. 2016). In the Briant et al. study, the peak cross-correlation between beat-to-beat MSNA burst area and DBP occurred at 7.44 ± 0.42 cardiac cycle, equating to a lag of 5.7–7.6 s at the mean heart rate for the cohort of 63 bpm (Briant, Burchell et al. 2016). This is longer than the baroreflex latency of ~1.2 s (Hart, Joyner et al. 2010) since mechanical transduction of the change in SNA into a change in vascular tone, and thus DBP, is required.

This gives a plot of DBP for each cardiac cycle versus MSNA burst area measured at the 8-6 beat lag. MSNA burst area (units of %•s) was binned into 1%•s bins, and the associated DBP (mean ± SEM) calculated. The slope of the weighted linear regression of the relationship between MSNA burst area and DBP, quantified sympathetic neurovascular transduction (units mmHg/%•s). A non-significant slope was not used as exclusion criteria for data, as this may reflect poor transduction (rather than a failure of the analytical method).

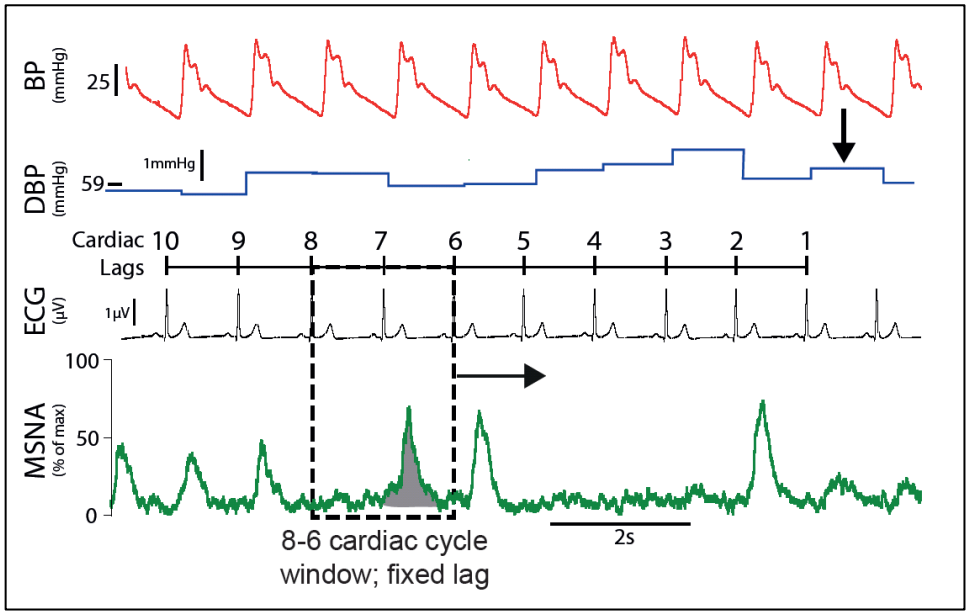


Figure 5-32. Method for quantifying the relationship between MSNA burst area and diastolic blood pressure (DBP): sympathetic neurovascular transduction.
For each DBP, MSNA burst are was measured at a lag of 8-6 cardiac cycles. MSNA burst are was then binned into 1 %•s bins and plotted against the mean DBP for that bin. The weighted slope of the relationship gives sympathovascular transduction. ECG; electrocardiogram, MSNA; muscle sympathetic nerve activity.

5.4.2.3 Results

Sympathovascular transduction was quantified in 13/18 participants at baseline (14 patients with MSNA, error with DBP recording in 1 participant therefore unable to calculate transduction). Baseline transduction ranged from -0.18 mmHg/%•s to 0.26 mmHg/%•s, with a more marked negative relationship (less transduction efficacy) between DBP and MSNA area (but similar maximum value) when compared with data in the normotensive population (Briant, Burchell et al. 2016).

There were no correlations between baseline sympathovascular transduction and baseline oSBP (see Figure 5-33), baseline MSNA incidence (see Figure 5-33), baseline total peripheral resistance (TPR; n=13, R=0.25, p=0.21), or any of the baseline cardiac or sympathetic BRS parameters, baseline HRV LF/HF ratio, any of the baseline cardiac volumetric and strain parameters, or versus baseline aortic distensibility (all p>0.05, see Appendix 2).

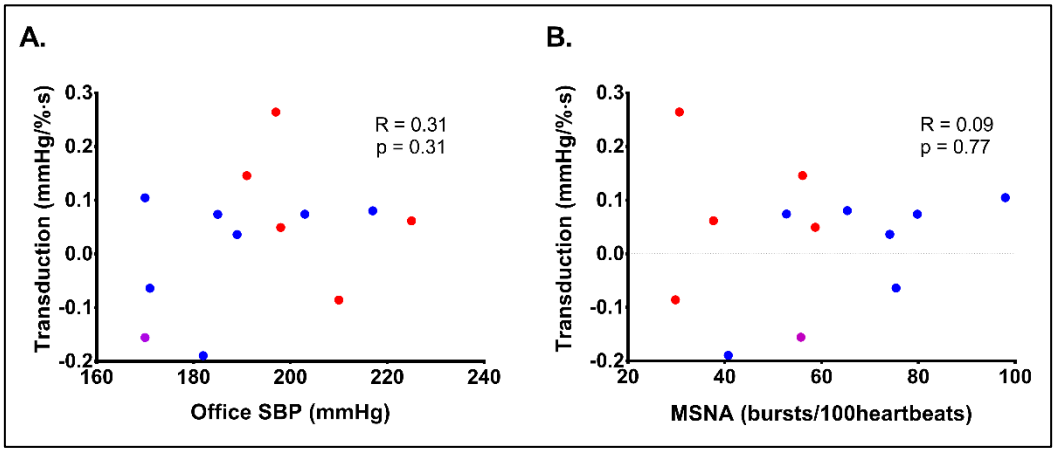


Figure 5-33. No correlations between baseline sympathovascular transduction and A. baseline office systolic blood pressure (SBP) and B. baseline muscle sympathetic nerve activity (MSNA).
Data for male participants is shown in blue, with data for premenopausal women in red and postmenopausal women in purple. This once again emphasises relatively low baseline MSNA, despite grossly elevated SBP, in the pre-menopausal participants. If the pre-menopausal participants are removed from the analysis, the relationship between baseline MSNA and baseline transduction is borderline significant (n=8, R=0.65, p=0.08), although this is not the case for the relationship versus baseline office SBP (n=8, R=0.46, p=0.25).

There were 10 participants with sympathovascular transduction data at baseline and the primary 6-month study follow-up visit. Amongst these subjects, there was no significant change in transduction at 6 months after denervation (0.05 ± 0.04 mmHg $(\%\bullet s)^{-1}$ vs 0.08 ± 0.02 mmHg $(\%\bullet s)^{-1}$, n=10, p=0.53). There was also no significant change in transduction following RDN when data were analysed by repeated-measures ANOVA over all study visits (see Table 5-23).

There were no correlations between the change in sympathovascular transduction at six months post-RDN versus the change in TPR (n=10, R=-0.13, p=0.73) or versus the changes in any of the other autonomic parameters assessed following RDN, including oSBP, MSNA and aortic distensibility, as shown in Appendix 3.

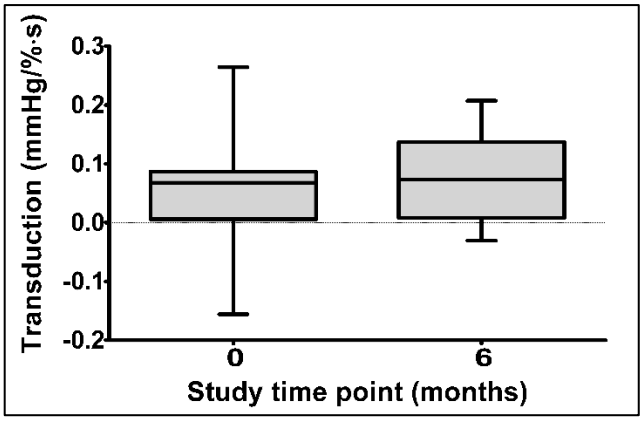


Figure 5-34. No change in sympathovascular transduction following renal denervation. There was no change in sympathovascular transduction amongst the 10 study participants with transduction data at baseline and at the 6-month primary study endpoint (as assessed by paired Student's t-test).

Parameter	Time post RDN (months)					P
	0	1	3	6	12	
Transduction (mmHg/%•s)	0.03 ± 0.03	0.02 ± 0.02	0.01 ± 0.04	0.07 ± 0.02	0.07 ± 0.03	0.39

Table 5-23. No change in sympathovascular transduction after renal denervation (RDN) over 12 months follow-up.
When data were analysed by repeated-measures ANOVA (data carried forward if gaps), there was no change in transduction over the full course of the study (Friedman test) with no significant differences on between group comparisons (Dunn's multiple comparison test). Data shown for the 13 participants with transduction data at baseline (raw data for n=13, 11, 8, 10 and 10 participants at 0, 1, 3, 6 and 12 months, respectively).

In summary, there was no change in sympathovascular transduction following RDN, and there were no correlations between either baseline sympathovascular transduction and the other baseline variables, or the change in sympathovascular transduction at 6 months post-RDN and changes in any of the other autonomic variables.

5.4.2.4 Discussion

In this, the first study to investigate the effect of renal denervation on sympathetic neurovascular transduction, there was no change in sympathovascular transduction following denervation (see Figure 5-34 and Table 5-23). There were also no correlations between any possible change in transduction post RDN and the changes in any of the other autonomic parameters following RDN. These results would suggest that sympathetic neuro-vascular transduction is not impacted by renal denervation, although further data are required to confirm these findings in a larger population. Sympathovascular transduction is affected by multiple factors, including co-transmitters acting pre-and post-synaptically, other local vasoactive agents and the relative

expression of α1- (vasoconstrictor) and β2- (vasodilator) adrenergic receptors (Guyton and Hall 1996, Burnstock 2008, Briant, Burchell et al. 2016). Furthermore, our participants are on a range of vasoactive medications, including α1-blockers, β-blockers and peripherally acting vasodilators such as long acting nitrates (see Appendix 1). The variable effects of these different agents are hard to quantify on an individual basis, and future studies should consider a standardized medication regime, or even an off-medication approach in subjects with less severe hypertension to enable more definitive evaluation of any effect of RDN on sympathetic neuro-vascular transduction.

It was also interesting to note that there were no correlations between baseline transduction and the other autonomic parameters quantified in this study at baseline, particularly versus MSNA incidence, TPR and BRS. It might have been expected that increased sympathovascular transduction was correlated with higher MSNA, greater BRS and particularly, increased TPR. The lack of any significant relationship between transduction and any of these variables, even at baseline, may reflect the small numbers in this cohort, and also the mix of male, and pre-menopausal and post-menopausal female participants. The factors affecting sympathovascular transduction are complex but appear to differ by age and gender. In women, sympathetic vascular coupling increases with age, being particularly low in pre-menopausal females. In contrast, in men, sympathovascular coupling decreases with age, meaning that in older men (>50 years) for a given level of MSNA there is less vasoconstriction, although the resting level of vasoconstriction must be taken into consideration, since further constriction may not be possible at high/maximal levels of vascular tone (Briant, Burchell et al. 2016). In premenopausal women sympathovascular transduction is low, and one mechanism for this is the high level of β2-adrenergic vasodilation in these younger females, this competes against the α1-adrenergic vasoconstriction, reducing transduction, as evidenced by a significant increase in sympatho-vascular coupling in response to systemic β-adrenergic blockade (Hart, Charkoudian et al. 2011, Briant, Burchell et al. 2016).

A limitation of these data is the method used to quantify transduction. DBP was used as a surrogate marker for the vascular response to MSNA, and whilst sympathetic vasomotor tone has been shown to correlate well with other measures, such as total peripheral resistance (TPR) (Barnes, Matzek et al. 2012, Briant, Burchell et al. 2016), direct measurement of vascular resistance may have been more specific. TPR was quantified from a composite of office BP and cardiac output derived from the volumetric CMR data. These measurements were not contemporaneous with the MSNA measured and were only available at baseline and 6 months post-RDN, and therefore did not provide a robust outcome measure for the quantification of sympathovascular transduction in this study. Reassuringly, previous data have shown a direct linear relationship between transduction into DBP and transduction into TPR (Briant, Burchell et al. 2016), and support the extrapolation of our findings.

5.4.3 Chemoreflex sensitivity

5.4.3.1 Introduction

Peripheral chemoreceptors, predominantly located in the carotid bodies, are activated by hypoxia, hypercapnia or increased hydrogen ion concentration, resulting in sympathoexcitation (Paton, Sobotka et al. 2013). Raised tonic chemoreceptor activity has been demonstrated in hypertensive humans (Sinski, Lewandowski et al. 2012, Pijacka, Moraes et al. 2016). Recurrent hypoxic exposures can increase chemosensitivity, which helps to explain the pathological relationship seen between the intermittent hypoxias of obstructive sleep apnoea and hypertension (Weiss, Liu et al. 2007). Increased chemosensitivity is also observed in the early stages of human hypertension, supporting a causal relationship (Trzebski, Tafil et al. 1982, Somers, Mark et al. 1988). There is now evidence that reducing chemoreflex mediated sympathoexcitation either through carotid body denervation or pharmacological inhibition in a rodent model, or via carotid body excision in humans, can have an antihypertensive effect (Abdala, McBryde et al. 2012, McBryde, Abdala et al. 2013, Narkiewicz, Ratcliffe et al. 2016, Pijacka, Moraes et al. 2016). Considering these findings, any intervention that reduces chemoreflex sensitivity may have a beneficial role in the management of hypertension, and we have therefore assessed the effect of RDN on chemoreflex sensitivity as quantified by the hypoxic ventilatory response (HVR).

5.4.3.2 Methods

In the initial part of the study peripheral chemoreflex sensitivity, as assessed by hypoxic ventilatory response, was assessed using an intermittent hypoxia technique; data for 10/18 participants were recording at baseline using this method. This method involves exposure of the patients to inspired nitrogen (N2) for 5-30 second bursts, resulting in a brief lowering of blood oxygen saturations (SpO2) for a few breaths at a time. During the course of the study, there were some concerns about this technique. It was possible for participants to breath hold during exposure to N2, and whilst the N2 was administered without prior warning, it was not imperceptible to the participants. It is also important to isolate the *peripheral* chemoreceptor response to hypoxia by maintaining normocapnia to avoid activation of the central chemoreceptors (Ciarka, Cuylits et al. 2006). In light of this, we switched to a stepped hypoxia method for the latter part of the study, which involved more prolonged exposure to two stepped increments of hypoxia. Data were collected for HVR using this stepped hypoxia approach in 6/18 participants at baseline. HVR data were not collected in 2/18 participants for logistical reasons. HVR was quantified at baseline and at 6 months after renal denervation.

5.4.3.2.1 Intermittent hypoxia method

As described previously (Chua and Coats 1995, Niewinski, Tubek et al. 2014, Narkiewicz, Ratcliffe et al. 2016), in this poikilocapnic hypoxic protocol, participants were switched from breathing room air to 100% N2 for increasing intervals of 5-30 seconds, with a 3 minute recovery period between each episode of hypoxia to allow physiological parameters to return to baseline. Each subject was exposed to 5-6 intervals of hypoxia to achieve a range of minimum SpO2 from 100% to 75%. Minute ventilation (MV) was

calculated from measures of breathing rate and tidal volume which were recorded continuously during the protocol using spirometry (AD Instruments, Sydney, Australia). For each hypoxic interval, MV for the largest 2 subsequent breaths during/following inhalation of 100% N2 was averaged. The HVR was quantified as the slope of the linear regression of MV plotted against the minimum SpO2 for each interval (see Figure 5-35).

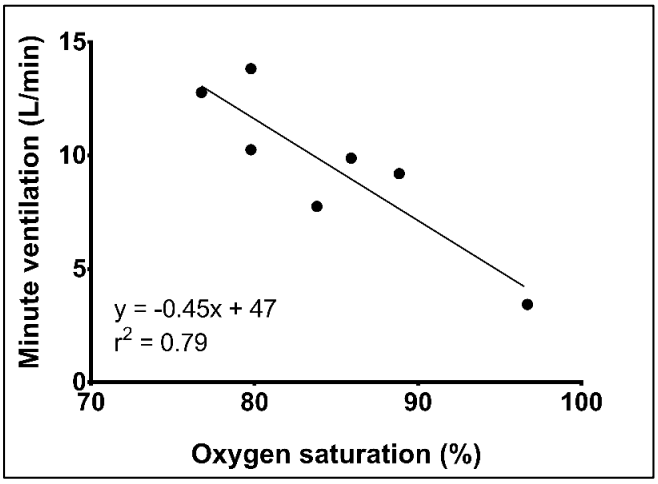


Figure 5-35. Example of the calculation of hypoxic ventilatory response (chemoreflex sensitivity) using the intermittent hypoxia protocol.
Data shown is baseline data for participant number 8. HVR is quantified as the slope of the linear regression of minute ventilation versus oxygen saturation plotted for each incremental burst of hypoxia.

5.4.3.2.2 Stepped hypoxia method

As similar to previously described methods (Ciarka, Cuylits et al. 2006, Breskovic, Valic et al. 2010), in this normocapnic hypoxic protocol, participants were stepped from breathing room air through two progressive steps of hypoxia by increasing the inspired N2 concentration until the target SpO2 was reached. Following a 1-minute baseline recording during exposure to room air, the target SpO2 for the first 2-minute step was 90-95% and the target SpO2 for the second 2-minute step was 87-90%. End tidal carbon dioxide (CO2) was maintained by titrating CO2 into the inspired gas. MV was calculated from measures of breathing rate and tidal volume, which were recorded continuously during the protocol using spirometry (AD Instruments, Sydney, Australia). For each of the three steps (1-minute baseline, 2 x 2-minute hypoxia) mean SpO2 and mean MV were calculated, and HRV was quantified as the regression line of the slope for the relationship of mean SpO2 versus mean MV from these three data points (see Figure 5-36).

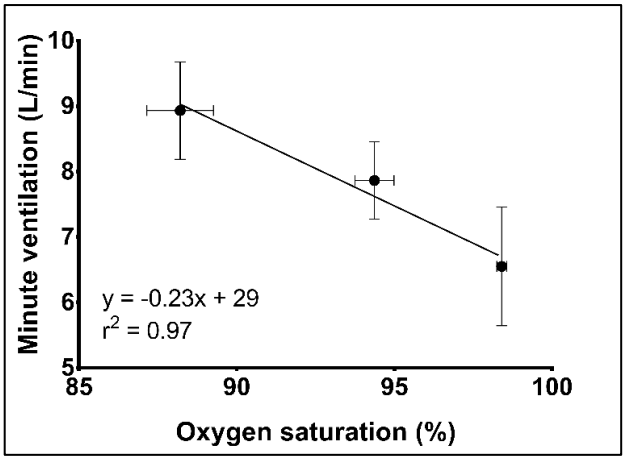


Figure 5-36. Example of the calculation of hypoxic ventilatory response (peripheral chemoreflex sensitivity) using the stepped hypoxia protocol.
Data shown is baseline data for participant number 18. HVR is quantified as the slope of the linear regression of minute ventilation versus oxygen saturation plotted for baseline oxygenation and each of the two progressive steps of hypoxia.

5.4.3.3 Results

Chemoreflex sensitivity was available in 16/18 participants, 10 assessed using the intermittent hypoxia method and 6 assessed using the stepped hypoxia regime.

At baseline there were no correlations between chemoreflex sensitivity (either using the intermittent hypoxia method the stepped hypoxia method, or for data from both methods combined) and office SBP, MSNA incidence or any of the other autonomic variables (see Appendix 2).

When looking at CMR measures of target organ damage, baseline chemoreflex sensitivity as assessed by the stepped hypoxia method, was negatively correlated against baseline ejection fraction (n=6, R=-0.88, p=0.02), baseline peak circumferential strain (n=6, R=-0.82, p=0.0048) and baseline peak diastolic circumferential strain rate (n=6, R=0.89, p=0.03). Greater chemoreflex gain was associated with a higher ejection fraction, but with lower circumferential strain and lower diastolic circumferential strain rate. There was a significant correlation between baseline HVR as assessed by the intermittent hypoxia method and baseline aortic distensibility (n=8, R=-0.76, p=0.03); individuals with greater chemoreflex sensitivity had greater aortic distensibility.

Amongst the participants with chemoreflex data available at both baseline and six months following RDN, there was no significant change in HVR, either for data for both HVR methods combined (n=12, -0.17 ± 0.06 L/min/%vs -0.14 ± 0.04 L/min/%, 0 and 6 months, respectively, p=0.91), or by the intermittent hypoxia method (n=8, -0.21 ± 0.07 L/min/% vs -0.18 ± 0.05 L/min/%, 0 and 6 months, respectively, p=0.84) or stepped hypoxia methods (n=4, -0.07 ± 0.11 L/min/% and -0.06 ± 0.04 L/min/%, 0 and 6 months, respectively, p=1.00, see Figure 5-37). Looking at Figure 5-37, chemoreflex sensitivity as assessed by the intermittent hypoxia method may give a greater HVR gain than the stepped hypoxia method, however, a direct comparison of these techniques within individual participants is required to confirm this observation.

Amongst the 12 patients who had chemoreflex HVR data at both baseline and six months following RDN, there were no significant correlations between the either the changes in office SBP, MSNA or sympathovascular transduction following RDN and the changes in HVR (see Appendix 3, note limited n for stepped hypoxia method)). The change in HVR post-RDN for patients assessed using the intermittent hypoxia method, correlated with the change in LF/HF ratio at 6 months after the procedure (n=7, R=0.78, p=0.04); an increase in relative sympathovagal balance was associated with a worsening of the HVR.

There was a correlation between the change in HVR as assessed with the intermittent hypoxia method and the change in overall spontaneous sBRST (n=7, R=-0.78, p=0.04). For this isolated variable, an increase in chemoreflex sensitivity was associated with a decreased in overall spontaneous sBRST, as reported previously there is an inhibitory interaction between the baroreflex and chemoreflex, with activation of the baroreceptors abolishing the SNA chemoreflex response to hypoxia (Somers, Mark et al. 1991).

There were correlations between the change in both overall HVR and HVR as assessed by the intermittent hypoxia method and the change in aortic distensibility following RDN (n=10, R=-0.72, p=0.02 and n=6, R=-0.95, p=0.004); an improvement in HVR following RDN was associated with an improvement in aortic distensibility, but there were also individuals with a decrease in HVR gain and a decrease in aortic distensibility, so whilst the correlation was strong, the directionality of the effect following RDN was mixed.

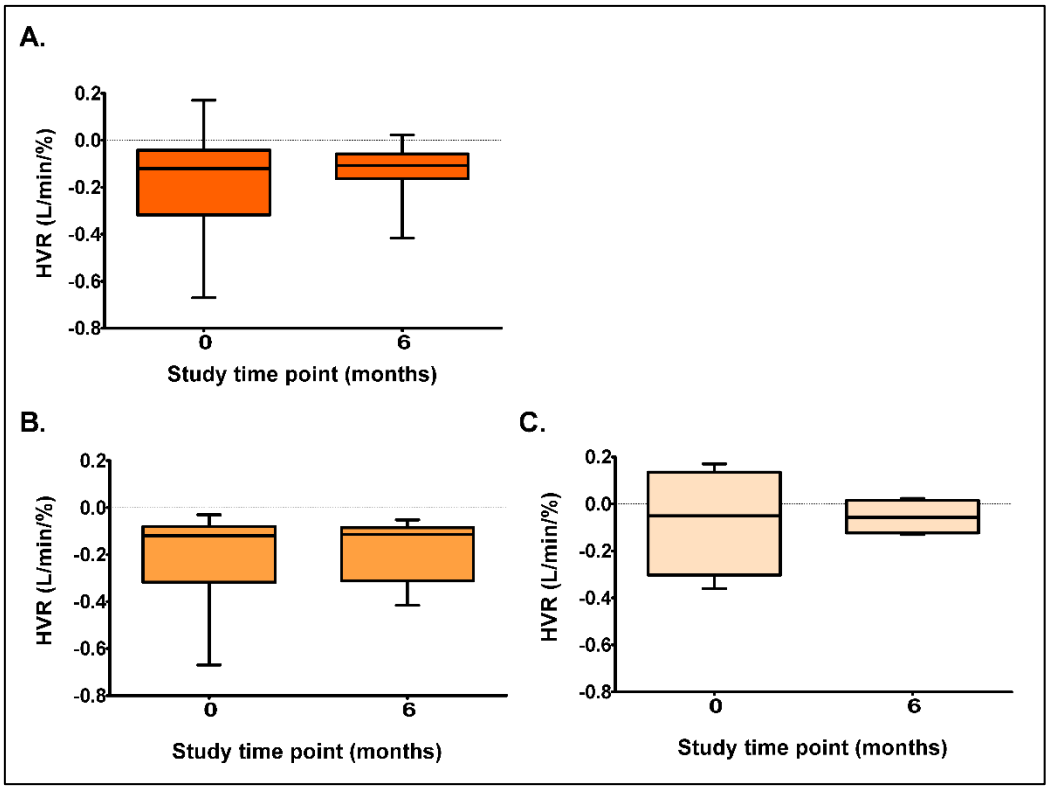


Figure 5-37. No change in chemoreflex as assessed by hypoxic ventilatory response (HVR) following renal denervation.

There was no change in chemoreflex for either A. all HVR data (n=12, p=0.91), or either B. HVR assessed by the intermittent hypoxia method (n=8, p=0.84) or c. HVR assessed by the stepped hypoxia method (n=4, p=1.00). Data assessed by Wilcoxon matched-pairs signed rank test.

In summary, there was no change in chemoreflex as assessed by the hypoxic ventilatory response following RDN. At baseline greater chemoreflex gain was associated with a higher ejection fraction, but with lower circumferential strain and lower diastolic circumferential strain rate, and individuals with greater chemoreflex sensitivity had greater aortic distensibility. Six months after denervation, an increase in relative sympathovagal balance was associated with a worsening of the HVR. An increase in chemoreflex sensitivity was associated with a decreased in overall spontaneous sBRST, and overall, an improvement in HVR following RDN was associated with an improvement in aortic distensibility.

5.4.3.4 Discussion

Regardless of the method used, there was no change in peripheral chemoreflex sensitivity as assessed by HVR following RDN. There were also no correlations between the different measures of HVR and any of the baseline autonomic variables. This may be surprising as patients with hypertension have been shown to have an increased sympathetic and ventilatory response to hypoxia (Somers, Mark et al. 1989, Kara, Narkiewicz et al. 2003, Sinski, Lewandowski et al. 2012). The chemoreflex mediates sympathoactivation and inhibition of baroreflex function (Paton, Sobotka et al. 2013), and conversely the arterial baroreflex also has a strong inhibitory effect on peripheral chemoreflex sensitivity, so it may have been expected to have seen a stronger relationship between these variables (Kara, Narkiewicz et al. 2003). However, in this small cohort of patients with resistant hypertension, the effect of gender on BRS and sympathovascular transduction, and extremes of BP and MSNA, may mask this relationship.

Interestingly, in this study a reduction in chemoreflex sensitivity was associated with an increase in sympathovagal balance and an increase in overall spontaneous sBRST. These results are rather conflicting since a decrease in chemoreflex sensitivity and an increase in BRS would be predicted to decrease SNA, with previous findings which established a sympathoexcitatory effect for chemoreflex activation (Kara, Narkiewicz et al. 2003, McBryde, Abdala et al. 2013, Paton, Sobotka et al. 2013). These findings were not consistent across all autonomic outcome measures, changes in which largely failed to correlate with changes in HVR following RDN. In a rat model, combined renal denervation and carotid sinus denervation (abolishing the afferent signal from the carotid body and therefore disrupting the chemoreflex feedback loop) have shown an independent and summative effect, suggesting different mechanisms of action for the antihypertensive effects of RDN and disruption of the peripheral chemoreflex (McBryde, Abdala et al. 2013), and therefore it is not surprising that RDN had no significant impact on chemoreflex sensitivity if these findings can be translated into human subject. In this, the first study to assess chemoreflex sensitivity following RDN in humans, the

interaction between RDN and chemoreflex sensitivity remains unclear, since patients had both increases and decreases in HVR following the intervention, and an inconsistent association with other autonomic variables.

It is clearly a limitation of this study that two different methods were used to assess HVR, since this makes it difficult to compare data for the full study cohort. The concerns over the intermittent hypoxia method are understandable, particularly the issues of breath-holding or hyperventilation during the bursts of nitrogen, and the fact that the test was poikilocapnic and so may also activate the central chemoreceptors. The stepped hypoxia aimed to achieve normocapnia and so more specifically isolated the peripheral chemoreflex response, however in most participants a lower level of hypoxia was achieved, which may explain the lower values for chemoreflex sensitivity (or HVR) when quantified by this method. Further research is required to directly compare these different techniques in normotensives and hypertensive subjects.

Finally, when looking at CMR measures of target organ damage, baseline chemoreflex sensitivity was associated with a higher ejection fraction, but with lower circumferential strain and lower diastolic circumferential strain rate. There were no correlations between any possible change in HVR and changes in cardiac volumetric or strain parameters following denervation. In a canine model, chemoreflex stimulation produced a positive inotropic response (Vatner and Rutherford 1978), conversely, chemoreflex sensitivity has also been shown to be increased in animal models of heart failure (Schultz, Marcus et al. 2013), reflecting the conflicting relationships between chemoreflex sensitivity and measures of cardiac function in this severely hypertensive cohort. Interestingly (given the largely negative outcomes with respect to aortic distensibility in the results described to date from this study), at baseline, individuals with greater chemoreflex sensitivity had greater aortic distensibility. Furthermore, an increase in HVR following RDN was associated with an increase in aortic distensibility, although equally, a decrease in HVR was associated with a decrease in aortic distensibility. There is no published data relating chemoreflex sensitivity and aortic distensibility (PubMed search chemoreflex sensitivity and aortic distensibility, or chemoreflex sensitivity and pulse wave velocity), so it is difficult to put these findings into context. However, increased sympathoexcitation from chemoreflex hypersensitivity would likely result in increased vascular tone and thus decreased aortic distensibility, and therefore further research is required to elucidate these findings and the underlying mechanisms.

5.4.4 Brain blood flow

5.4.4.1 Introduction

The peripheral chemoreceptors, located in the carotid bodies, are ideally placed to monitor and maintain the supply of oxygenated blood to the brain. Beyond this chemoreflex, under the ‘Selfish Brain Hypothesis’, the brain will act to maintain cerebral perfusion and oxygenation, even at possible detriment to systemic arterial pressure and

at the expense of other organs (see Section 2.1.2.3.1) (Paton, Dickinson et al. 2009, Cates, Dickinson et al. 2012, Hart 2016). Vertebral artery hypoplasia (VAH), a congenital vascular variant, and increased cerebrovascular resistance (CVR) have been demonstrated to have a possible causal effect on hypertension (Warnert, Rodrigues et al. 2016). In this study we have assessed cerebrovascular anatomy and quantified cerebral blood flow (CBF) using MRI. We have hypothesised that individuals with high cerebral artery resistance and hence cerebral hypoperfusion, driving increased sympathetic nerve activity would be more likely to respond to the potential sympathoinhibitory effect of RDN. This is the first study to investigate the impact of RDN on CBF and will provide useful pilot data to guide further research in the field.

5.4.4.2 Methods

Cerebral angiography was performed using 3-Dimensional time-of-flight magnetic resonance angiography (MRA) at 1.5T (Avanto, Siemens, Erlangen, Germany) with a dedicated head coil used to measure arterial anatomy (repetition time=38 ms, echo time=5.28 ms, flip angle=25°, voxel size=0.7×0.5×0.8 mm^3, field of view=200 mm, covering major arteries feeding into the Circle of Willis (CoW)). VAH was defined as a diameter <2 mm uniformly throughout the vessel.

Cerebral blood flow was quantified using through plane phase contrast sequences (Siemens, Erlangen, Germany); repetition time=48.65 ms, echo time=2.15 ms, base resolution 192, 1.7x1.7x5.5 mm^3 matrix, GRAPPA (generalised autocalibrating partially parallel acquisition) Acceleration Factor: PE 2, velocity encoding=100 cm/s. The image slice was positioned perpendicularly to the carotid and vertebral vessels. Where the neck vessels were serpiginous, the sequence was repeated in a different anatomical position so that all four vessels were imaged correctly. The region of interest was drawn onto each vessel using phase and magnitude images and propagated through all phases of the series with manual adjustment to the vessel as required. Flow in each vessel was then calculated using specialist software (cvi42; Circle Cardiovascular Imaging Inc., Calgary, Canada). Flow in each vessel was also quantified as a percentage of overall cerebral vessel, and as a percentage of cardiac output (CO).

CBF parameters were correlated against other measures of autonomic function as baseline and for 6-month post-RDN outcomes.

5.4.4.3 Results

Cerebrovascular MRI was performed in 16/18 participants at baseline. Of these 16 participants 50% had congenital cerebral vascular abnormalities; 4 patients with vertebral artery hypoplasia, and 4 participants with an incomplete circle of Willis (see example in Figure 5-38).

At baseline, there were no significant correlations between either office SBP, MSNA incidence, LF/HF ratio, sympathovascular transduction, spontaneous sBRST (overall), spontaneous sBRSA (overall), BEI, or chemoreflex (overall, intermittent hypoxia HVR and stepped hypoxia HVR), and any of the CBF parameters (all $p>0.05$).

CBF data were available in 14 participants at 6 months after renal denervation, amongst these individuals there were no significant changes in any of the CBF variables between baseline and 6 months after RDN (see Table 5-24). When data were analysed by RDN BP response group, there were no significant changes in any of the CBF parameters amongst either RDN responders or RDN non-responders, and no significant difference between the changes in any of the CBF parameters between response groups.

There were no significant correlations (n≥5) between the changes in office SBP, overall spontaneous sBRST or sBRSA, overall spontaneous cBRS, overall BEI, LF/HF HRV spectral power, or measures of HVR and any of the CBF parameters (all p>0.05). The change in MSNA incidence 6 months post-RDN correlated with the changes in total cerebral blood flow (n=9, R=0.75, p=0.02, see Figure 5-39).

Parameter	**Time post-RDN (months)**		
	0	6	P
RCA (ml/min)	436 ± 33	385 ± 28	0.15
LCA (ml/min)	414 ± 20	396 ± 32	0.48
RVA (ml/min)	125 ± 11	126 ± 10	0.94
LVA (ml/min)	160 ± 16	149 ± 14	0.28
Total cerebral flow (ml/min)	1135 ± 63	1056 ± 73	0.25
% RCA flow of cerebral flow	38 ± 1.3	37 ± 0.9	0.19
% LCA flow of cerebral flow	37 ± 1.1	37 ± 0.7	0.79
% RVA flow of cerebral flow	11 ± 0.8	12 ± 0.9	0.13
% LVA flow of cerebral flow	46 ± 18	14 ± 0.8	0.43
% cerebral flow of CO	17 ± 1.6	16 ± 1.1	0.45
% RCA of CO	6 ± 0.6	6 ± 0.4	0.34
% LCA of CO	6 ± 0.6	6 ± 0.5	0.63
% RVA of CO	2 ± 0.2	2 ± 0.2	0.80
% LVA of CO	2 ± 0.3	2 ± 0.2	0.42

Table 5-24. No changes in cerebral blood flow following renal denervation (RDN). There were no changes in either total cerebral blood flow or blood flow in each of the four cerebral arteries, either when expressed as absolute values in ml/min, or when expressed as the percentage of total cerebral blood flow measured in each of the arteries, or as a percentage of overall cardiac output (CO). RCA; right carotid artery, LCA; left carotid artery, RVA; right vertebral artery, LVA; left vertebral artery.

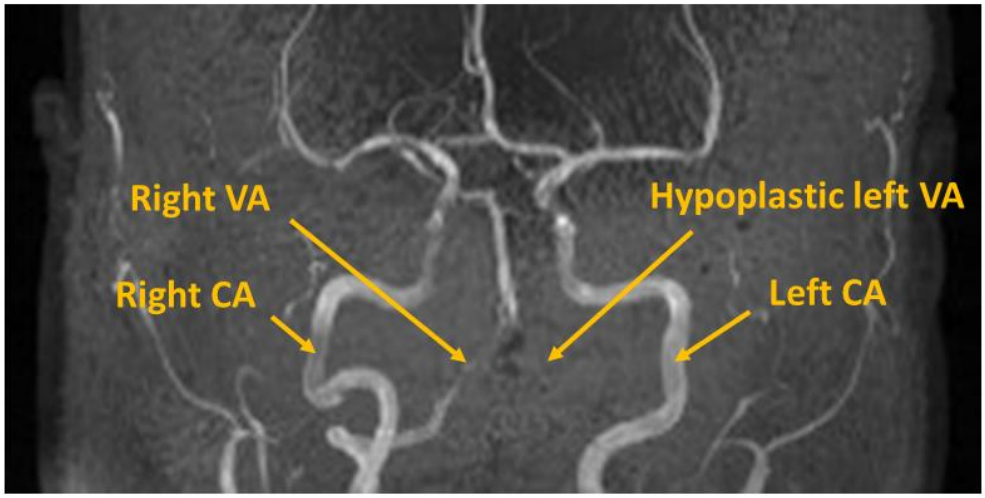


Figure 5-38. Example of magnetic resonance cerebral angiogram showing a hypoplastic left vertebral artery.
VA; vertebral artery, CA; carotid artery.

When considering the relationship between CBF and other cardiovascular parameters, there was a correlation between baseline iLVM and baseline total CBF as a percentage of cardiac output n=15, R=0.55, p=0.04, see Figure 5-39); higher relative CBF was associated with a higher iLVM.

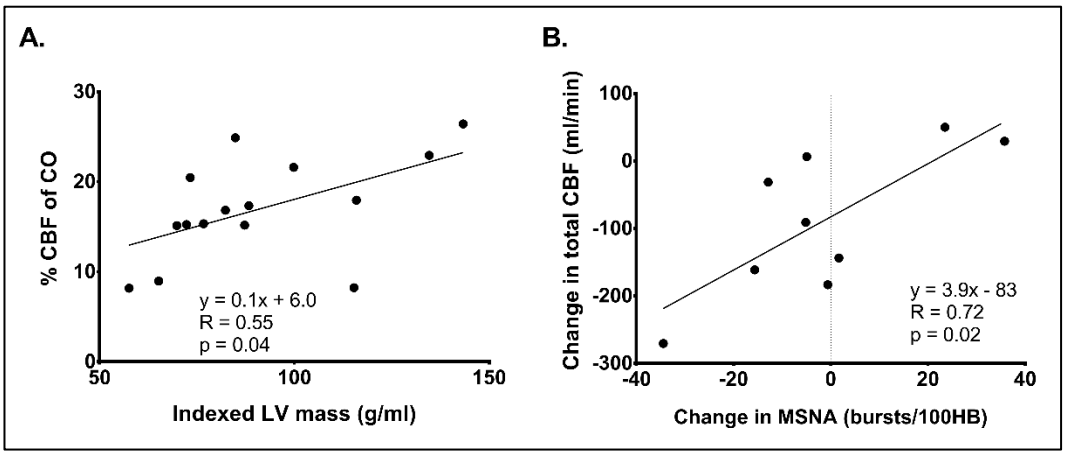


Figure 5-39. Correlations between A. baseline left ventricular mass index and baseline cerebral blood flow (CBF) as a percentage of cardiac output (CO) and B. the changes in muscle sympathetic nerve activity (MSNA) incidence and the changes in total cerebral blood flow at six months after renal denervation.

5.4.4.4 Discussion

There was no change in cerebral flow following RDN, including no change in absolute CBF or CBF relative to cardiac output, and no change in the flow in the individual carotid or vertebral arteries (see Table 5-24). There was also no difference in the effect of RDN on CBF between RDN BP response groups.

The change in MSNA incidence at 6 months after RDN correlated with the change in CBF following the intervention (see Figure 5-39), with a reduction in SNA being associated with a reduction in CBF. This may suggest that CBF is maintained by increased peripheral vascular tone, and thus systemic BP in these individuals (Warnert, Rodrigues et al. 2016), however, this relationship did not hold true when CBF was quantified as a percentage of total cardiac output.

Strikingly, 50% of the study participants had congenital cerebrovascular abnormalities, consisting of vertebral artery hypoplasia and/or an incomplete circle of Willis. The prevalence of cerebrovascular abnormalities in this cohort of patients with resistant hypertension is significantly greater than the ~30% prevalence seen in the normotensive population (Warnert, Rodrigues et al. 2016), but not dissimilar to the higher prevalence of these variants in patients with established hypertension (VAH 53% prevalence and incomplete posterior circle of Willis 64% prevalence (Warnert, Rodrigues et al. 2016)). Notably, CBF did not correlate with oSBP or MSNA incidence at baseline.

When considering the relationship between CBF and cardiovascular end organ damage, higher relative cerebral blood flow was associated with a higher iLVM in this hypertensive cohort prior to RDN (see Figure 5-39). This may be an example of hypertension preserving cerebral perfusion at the detriment of cardiac hypertrophy and vascular stiffness, but further research is required to confirm these observations in a larger population and to establish causality.

There are limited data available on the impact of RDN on CBF. In a small case-control study, Efimova et al. report an improvement in regional CBF following RDN, which was also related to improvements in cognitive function (abstract reviewed only, in Russian (Efimova, Lichikaki et al. 2017)). This is interesting, since congenital cerebrovascular variants and increased cerebrovascular resistance have been associated with the development of hypertension, raising the question of whether treating hypertension could reduce cerebral perfusion (Warnert, Rodrigues et al. 2016).

5.4.5 Markers of inflammation

5.4.5.1 Introduction

Systemic and central inflammation have been increasingly seen to play an important role in the development of hypertension. Levels of systemic inflammatory markers such as such as tumour necrosis factor alpha (TNFα), interleukin-6, C-reactive protein (CRP) and adhesion molecules are increased in hypertension and may have a pro-hypertensive role (Fisher and Paton 2012, Marvar, Vinh et al. 2012). Systemic inflammation can upregulate microglia, pro-inflammatory cytokines and reactive oxygen species within the rostral ventrolateral medulla, with an associated rise in blood pressure and increased sympathetic vasomotor tone (Wu, Chan et al. 2012). Treatment with anti-inflammatory drugs has been shown to reduced BP in animal models (Zhang, Wei et al. 2003, Wu, Chan et al. 2012), and in humans the angiotensin receptor blocker valsartan has been shown to reduce both arterial pressure and levels of the pro-inflammatory cytokines TNFα and IL-6. The role of inflammation, particularly central inflammation, in driving

high blood pressure may therefore represent a novel therapeutic target for the clinical management of hypertension. The primary activities of the inflammatory markers quantified in this study are summarised in Table 5-25.

As well as evidence for a pro-hypertensive role for systemic and central inflammation, activation of the sympathetic nervous system itself is proinflammatory (Singh, Chapleau et al. 2014), we hypothesis that a reduction in BP following RDN would be associated with a reduction in systemic inflammation, and that patients with raised markers of inflammation would be more likely to respond to RDN with a reduction in BP.

5.4.5.2 Methods

Blood samples were taken from the study participants at each study visit. The samples were taken after 5 minutes sitting at rest at room temperature. Samples for standard clinical measures, particularly blood to assess the safety of the procedure (e.g. renal function), were assessed through standard clinical pathways at University Hospitals Bristol NHS Foundation Trust. Samples for analysis of systemic inflammatory markers, were processed to obtain anonymised serum samples which were then stored at -70 ^{o}C in a locked facility for practical reasons, to enable bulk analysis. Unfortunately, systems for the processing and storage of these samples took time to put in place, and samples for inflammatory marker analysis were not stored for the first eight participants in the study.

CRP was quantified in the Clinical Pathology Department of the Bristol Royal Infirmary (University Hospitals Bristol NHS Foundation Trust). From the stored serum samples, we quantified levels of IL-6, IL-8, IL-10, IL-17, TNFα and myeloperoxidase (MPO). A Luminex system (Luminex Corporation, Austin, Texas, USA) was used for the quantification of these cytokines, this flow cytometry-based system uses magnetic microspheres (Milliplex, Merck, Darmstadt, Germany). conjugated to specific capture antibodies to quantify multiple biomarkers from a single ~1 mL serum sample. All Luminex assays were performed by Dr Tanya Smith under the supervision of Dr Saadeh Suleiman, who were blinded to the study BP outcome.

Inflammatory marker	Principle source	Primary activities
CRP	Secreted by liver in response to cytokines release by macrophages and NK cells (e.g. IL -6)	Acute phase protein: binds to lysophosphatidylcholine expressed on the surface of dead or dying cells to activate complement system
IL-6	Activated Th2 cells, APCs, macrophages, other somatic cells	Pro-inflammatory: Acute phase response/fever, lymphocyte activation
IL-8	Monocytes, macrophages, fibroblasts, keratinocytes	Chemokine: chemoattractant for neutrophils and T cells
IL-10	Activated Th2 cells, CD8+ T and B cells, macrophages	Anti-inflammatory: inhibits cytokine production, promotes B cell proliferation and antibody production,

		suppresses cellular immunity and macrophage activity
IL-17	Th17 cells	Pro-inflammatory: activates induction of IL-6, IL-8 and G-CSF amongst others
TNFα	Macrophages, mast cells, NK cells, sensory neurons	Pro-inflammatory: cell death, inflammation, fever, shock, pain, activates vascular endothelium, increases vascular permeability
MPO	Activated neutrophils and macrophages	Pro-oxidant enzyme which breaks down microorganisms

Table 5-25. Summary of the principle activities of the inflammatory markers assessed in this study.
CRP; C-reactive protein, NK; natural killer, IL; interleukin, Th; T-helper cells, G-CSF; granulocyte colony stimulating factor, TNFα; tumour necrosis factor-alpha, MPO; myeloperoxidase (Lydyard, Whelan et al. 2000, Zhang and An 2007, Gaffen 2008, Heslop, Frohlich et al. 2010).

5.4.5.3 Results

CRP was quantified in 9 subjects at baseline, and 8 subjects had data available at baseline and the primary study outcome timepoint of 6 months post-RDN. The other inflammatory markers were assessed in a slightly different subset of 9 study participants, with data available in all 9 participants at all study timepoints.

At baseline, there were no correlations between oSBP, MSNA incidence, sympathovascular transduction, LF/HF ratio, spontaneous sBRST (overall), spontaneous sBRSA (overall) or BEI (overall) and baseline levels of CRP, IL-6, IL-8, IL-10, IL-17, MPO or TNFα (all $p>0.05$). There was a significant correlation between baseline MPO levels and baseline spontaneous cBRS ($n=9$, $R=0.86$, $p=0.003$); higher MPO levels were associated with higher spontaneous cardiac baroreflex gain. Baseline overall chemoreflex (data pooled from both intermittent and stepped hypoxia methods) was correlated versus baseline CRP ($n=8$, $R=0.79$, $p=0.03$), and baseline chemoreflex (as assessed by the intermittent hypoxia method) was correlated with baseline MPO levels ($n=4$, $R=-0.97$, $p=0.03$); increased chemoreflex sensitivity was associated with a lower CRP but with a higher MPO level.

There were no significant changes in any of the inflammatory markers following RDN as assessed by paired t-test at baseline versus 6 months post-RDN, although there was a trend towards an increase in IL-8 following denervation (see Table 5-26). When data were assessed by repeated measures ANOVA across the full study follow-up period the rise in IL-8 attained significance, with significant differences in IL-8 at 1 and 12 months after the procedure (see Table 5-27 and Figure 5-40).

Parameter	**Time post RDN (months)**		**P**
	0	6	
CRP (mg/mL)	3.1 ± 0.6	3.0 ± 0.7	1.00

IL-6 (pg/mL)	3.5 ± 0.9	3.7 ± 0.7	0.55
IL-8 (pg/mL)	5.9 ± 2.1	7.0 ± 2.6	0.07
IL-10 (pg/mL)	9.1 ± 1.6	13.5 ± 2.6	0.16
IL-17 (pg/mL)	10.7 ± 2.3	11.6 ± 1.6	0.36
MPO (pg/mL)	42.5 ± 8.1	33.6 ± 4.3	0.42
TNFα (pg/mL)	9.1 ± 1.6	13.5 ± 2.6	0.16

Table 5-26. No change in inflammatory markers following renal denervation (RDN) at 6-month follow-up.

Data are for n=9 participants. The subset of patients with CRP data at baseline and 6 months differs slightly from the subset of patients in which the other inflammatory markers were assessed. Data analysed by paired Student's t-test or by Wilcoxon matched-pairs signed rank test (IL-8 data only). The data for IL-8 approaches a significant rise at six months after the procedure. CRP: C reactive protein, IL; interleukin, MPO; myeloperoxidase, TNFα; tumour necrosis factor alpha.

At 6 months after renal denervation, there was a correlation between the change in IL-8 and the change in oSBP (n=9, R=-0.73, p=0.03, see Figure 5-41), with a reduction in SBP associated with an increase in IL-8, but there were no other correlations between changes in oSBP and changes in other inflammatory markers (all p>0.05). There was a correlation between the change in IL-17 and the change in MSNA incidence at 6 months post-RDN (n=4, R=0.99, p=0.04, see Figure 5-41); a decrease in IL-17 was associated with a decrease in MSNA, but these data were only from a small subset of patients.

Parameter	**Time post RDN (months)**					**P**
	0	1	3	6	12	
CRP (mg/mL)	3.1 ± 0.6	3.1 ± 0.8	3.3 ± 0.7	3.1 ± 0.6	3.2 ± 0.7	0.99
IL-6 (pg/mL)	3.5 ± 0.9	4.6 ± 1.0	3.9 ± 0.7	3.7 ± 0.7	3.8 ± 0.7	0.51
IL-8 (pg/mL)	5.9 ± 2.1	7.1 ± 2.0*	7.2 ± 2.5	7.0 ± 2.6	10.0 ± 4.2*	0.03
IL-10 (pg/mL)	9.1 ± 1.6	11.5 ± 1.5	13.8 ± 1.8	13.5 ± 2.6	9.9 ± 1.4	0.09
IL-17 (pg/mL)	10.7 ± 2.3	14.6 ± 2.6	11.4 ± 1.5	11.6 ± 1.6	10.9 ± 1.6	0.11
MPO (pg/mL)	42.5 ± 8.1	31.7 ± 5.2	30.9 ± 4.4	33.6 ± 4.3	34.1 ± 8.7	0.59
TNFα (pg/mL)	9.1 ± 1.6	11.5 ± 1.5	13.7 ± 1.8	13.5 ± 2.6	9.9 ± 1.4	0.10

Table 5-27. Changes in inflammatory markers following renal denervation over the 12-month study duration.

Data are for n=9 participants. Data analysed by repeated measures ANOVA, data carried forward from previous visit to address gaps in CRP data, but data available at all time points for all other inflammatory markers. There was a significant increase in IL-8 over the course of the study. *Significant difference versus baseline data by Dunn's multiple comparison test (p<0.05). CRP: C reactive protein, IL; interleukin, MPO; myeloperoxidase, TNFα; tumour necrosis factor alpha.

At baseline, there were no correlations between either iLVM or total CBF (either absolute values or as percentage of CO) and the inflammatory markers (all p>0.05), however, baseline aortic distensibility was correlated against baseline IL-8 levels (n=9, R=0.73, p=0.03), with higher distensibility associated with higher IL-8 levels.

There was a significant correlation between the change in IL-17 and the change in iLVM at 6 months after denervation (n=9, R=0.73, p=0.02, see Figure 5-41); a decrease in IL-17 was associated with a decrease in iLVM. There was a significant correlation between the change in MPO and the change in aortic distensibility after the procedure (n=9, R=0.71, p=0.03, see Figure 5-41); a decrease in MPO was associated with a decrease in aortic distensibility. There were no significant correlations between changes in iLVM and aortic distensibility and any other changes in inflammatory markers (all p>0.05).

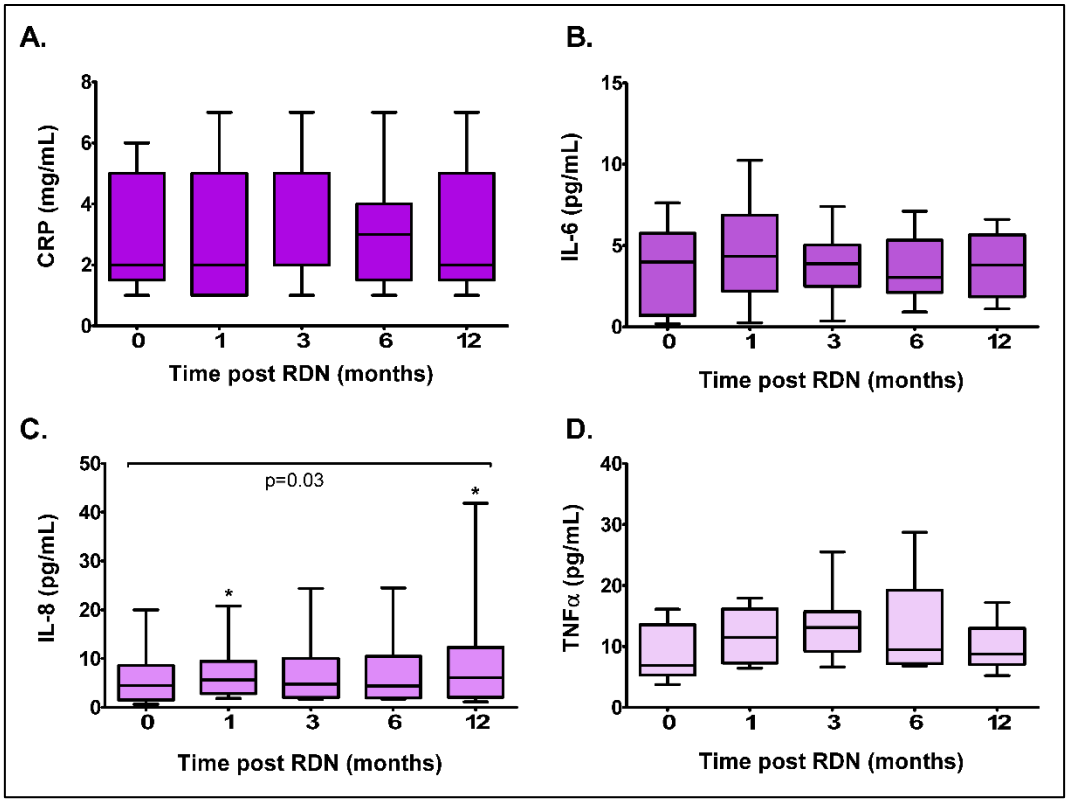


Figure 5-40. Changes in A. CRP, B. IL-6, C. IL-8 and D. TNFα following renal denervation (RDN).
Data are for n=9 participants. Data analysed by repeated measures ANOVA, data carried forward from previous visit to address gaps in CRP data, but data available at all time points for all other inflammatory markers. There was a significant increase in IL-8 over the course of the study. *Significant difference versus baseline data by Dunn's multiple comparison test (p<0.05). CRP: C reactive protein, IL; interleukin, MPO; myeloperoxidase, TNFα; tumour necrosis factor alpha.

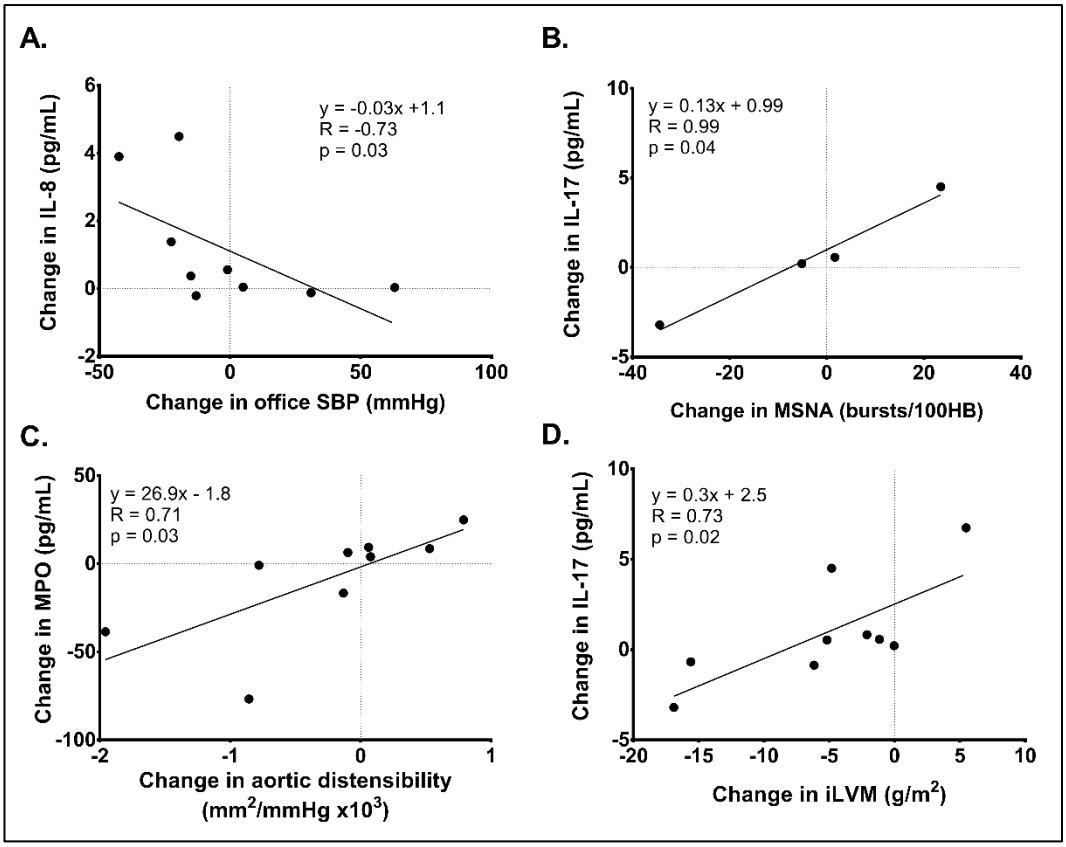


Figure 5-41. Correlations between changes in measures of cardiovascular parameters and inflammatory markers 6 months after renal denervation.
There were significant correlations between A. the change in office systolic blood pressure (SBP) and the change in IL-8 (interleukin-8), B, the change in muscle sympathetic nerve activity (MSNA) and the change in IL-17, C. the change in aortic distensibility and the change in myeloperoxidase (MPO), and D. the change in left ventricular mass index (iLVM) and the change in IL-17 after renal denervation.

5.4.5.4 Discussion

When considering all aspects of the data, whilst there were no changes in the majority of inflammatory markers over the course of this study, there was an increase in IL-8 following renal denervation. This goes against our hypothesis that pro-inflammatory cytokines would fall due to a reduction in pro-inflammatory sympathetic nerve activity following the procedure. There was no reduction in MSNA following RDN in this cohort, but this rise in IL-8 was associated with a reduction in office SBP (see Figure 5-41). Interestingly, there was a correlation between the change in MSNA incidence and the change in pro-inflammatory IL-17 following RDN, with a decrease in IL-17 was associated with a decrease in MSNA, but these data was only from a small subset of 4 patients.

Baseline CRP levels were largely within the normal range (<5 mg/mL, local reference range). Baseline IL-6, IL-8, IL-17, MPO and TNFα levels were lower than previously published normal range data, where-as IL-10 levels were within the normal range (Heslop, Frohlich et al. 2010, Biancotto, Wank et al. 2013, Kleiner, Marcuzzi et al. 2013). It is also notable that at baseline, there were no correlations between oSBP, MSNA

incidence, or spontaneous sympathetic baroreflex sensitivity, sympathovagal balance as assessed by LF/HF HRV ratio and sympathovascular transduction, and baseline levels of the inflammatory markers assessed in this study. Baseline MPO levels were correlated both with baseline spontaneous cardiac BRS and baseline chemoreflex (intermittent hypoxia method), with a higher level of MPO associated with higher spontaneous cBRS and higher chemoreflex sensitivity, which are conflicting findings for these inhibitory and excitatory reflexes, respectively. Baseline overall chemoreflex (data pooled from both intermittent and stepped hypoxia methods) was correlated versus baseline CRP, with a lower CRP associated with higher chemoreflex gain, which once again is counter to our hypothesis, and there are no published data on the relationship between MPO and either chemoreflex or cardiac baroreflex sensitivity it is difficult to further rationalise these findings.

At baseline aortic distensibility was correlated against baseline IL-8 levels, with higher distensibility associated with higher IL-8 levels, but there was no correlation between the change in aortic distensibility and the change in IL-8. There was a significant correlation between the change in aortic distensibility and the change in MPO after the procedure (Figure 5-41), with a decrease in MPO was associated with a decrease in aortic distensibility, again countering our hypothesis. A decrease in IL-17 was associated with a decrease in iLVM (see Figure 5-41), which would be more in keeping with cardiovascular benefits from a reduction in inflammation, and previous findings that IL-17A levels are higher in patients with target organ damage, including increased iLVM (Ates, Ozkayar et al. 2014).

These results are obviously difficult to interpret give the conflicting pro- and anti-inflammatory changes seen, and the lack of a consistent pattern of results in relation to baseline and 6-month changes in autonomic and target organ damage parameters. Numbers are small, but the increase in IL-8 over the course of the study is an interesting observation. Hypertensive patients have been shown to have increased levels of IL-8 (Marek-Trzonkowska, Kwieczynska et al. 2015), so it is notable that a reduction in BP was associated with a rise in IL-8 following RDN. It is also difficult to explain the association between higher levels of IL-8 and increased aortic distensibility. IL-8 has been shown to participate in the pathogenesis of hypertension; spontaneously hypertensive rats have increased levels of this chemokine, and angiotensin II has been shown to induce the expression of IL-8, an effect that is inhibited by the angiotensin receptor blocker losartan (Martynowicz, Janus et al. 2014). Furthermore, IL-8 plays an important role in the migration of leukocytes into the sub-endothelial vascular wall in the early stages of atherosclerosis, and is associated with a higher risk of coronary heart disease (Boekholdt, Peters et al. 2004), making it particularly surprising that higher IL-8 levels are associated with reduced vascular stiffness in this cohort. One explanation could be that a reduction in systemic BP following RDN may lead to relative hypoxia in some tissues which then stimulates IL-8 production. Any potential relationship between MPO levels and aortic distensibility also warrants further investigate as there are no published data on this topic.

Investigators have previously considered the effect of RDN on inflammatory markers. Some studies have shown no effect of RDN on inflammatory markers (Alexander, Johannes Kepler University - Linz General Hospital et al. 2015), whereas Dorr et al. reported reductions in SBP, IL-6 and CRP following RDN in a cohort of 60 patients with

resistant hypertension (Dorr, Liebetrau et al. 2015). Esler's group in Melbourne demonstrated no change in CRP, TNF-α, IL-6 or sICAM-1 (soluble intracellular adhesion molecule-1), although there was a small increase in the inflammatory marker sVCAM-1 levels (soluble vascular cell adhesion molecule-1), but comment on the fact that their patients have levels of inflammatory markers within the normal published range (Eikelis, Hering et al. 2015). In a further publication, Eikelis et al. also reported increases in sVCAM-1, and VGEF-A (vascular endothelial growth factor A), and a reduction in sVEGFR-1 (soluble vascular endothelial growth factor-1) following RDN[4], however these changes were observed in both RDN BP responders and non-responders (Eikelis, Hering et al. 2017). Zaldivia et al. reported a reduction in BP following RDN, as well as a reduction in monocyte activation, plasma monocyte chemoattractant protein-1 levels, IL-1β, TNFα, and IL-12 following RDN; in this study there was a positive correlation between MSNA and monocyte activation before and after the procedure (Zaldivia, Rivera et al. 2017).

Animal studies have also investigated the levels of renal cytokines before and after RDN. In a murine model, there were no changes in renal levels of the proinflammatory cytokines IL-1β, IL-2, IL-6, IL-17, TNFα, and interferon-γ, or the anti-inflammatory cytokine IL-10 following RDN (Asirvatham-Jeyaraj, Fiege et al. 2016), likewise there were no changes in renal inflammatory markers after RDN, despite a decrease in BP, in a deoxycorticosterone acetate (DOCA)-salt hypertension model in rats (Banek, Gauthier et al. 2018). In contrast, in a murine model of angiotensin-II-induced hypertension, renal denervation reduced the accumulation of both CD4+ and CD8+ T cells in the kidney as well as the production of IL-1α, IL-1β ad IL-6 from splenic dendritic cells (Osborn, Hana et al. 2015).

Baseline levels of the inflammatory markers were relatively low in this study, which may explain the absence of a consistent anti-inflammatory effect of RDN. The data in this field remain highly variable, and any anti-inflammatory effect of RDN remains to be established, along with any potential mechanism (including a reduction in SNA) which may underlie a reduction in inflammation. There are also logistical questions about the timing and duration of follow-up for any inflammatory changes and the selection of cytokines to be investigated from the vast array of available markers. In this cohort there were financial limitations impacting the number of cytokines which could be investigated, as well as technical issues regarding the markers that were compatible within a single Multiplex array. Antihypertensive medications, including angiotensin receptor blockers and spironolactone, have been shown to reduce inflammatory markers in hypertension (Singh, Chapleau et al. 2014), and the varying medication regimes within this cohort may also confound outcome data. All of these factors should be taken into consideration when considering further research in this field.

[4] sVEGFR-1 binds VEGF reducing its circulating levels. VEGF may have a vascular protective role through its actions on nitric oxide (NO); changes in the sVEGFR-1/VEGF ratio may increase NO bioavailability, increasing vasodilatation and reducing BP.

5.4.6 Conclusions

In this chapter I have reported the results of a wide range of physiological measures in subjects with resistant hypertension, following treatment with renal nerve ablation. These data illustrate both the positive aspects and challenges seen in this pilot study.

I have demonstrated that it is safe and feasible to measure a broad profile of autonomic parameters over the course of a longitudinal study in a severely hypertension population. I have been able to develop an armoury of research tools that can be applied to future projects. In some instances, further research is required to establish directly comparable, normal range data for these variables, and a case-control design would help to clarify outcomes. We have also seen inconsistent, and in some cases contradictory, outcomes across different autonomic variables. The measurement of multiple outputs from the assessments of HRV, BRS, CBF and their relationship to a range of measures target organ damage, makes it difficult to place great emphasis on an isolated finding in a single 'sub-variable' which may be influenced by the small size of the study cohort.

The more robust findings from these data can be summarised as follows:

1. There were no changes in sympathetic BRS, sympathovascular transduction, chemoreflex sensitivity, cerebral blood flow and the majority of the markers of inflammation measured in this study, following RDN.
2. There were no correlations between the either the change in office SBP or the change in MSNA, and the changes in BRS and chemoreflex sensitivity, at 6 months post-RDN.
3. At baseline, there were no correlations between either sympathovascular transduction or chemoreflex sensitivity measures and any of the other autonomic parameters quantified in this study.
4. At baseline, there were no correlations between levels of inflammatory markers and oSBP, MSNA incidence, spontaneous sympathetic baroreflex sensitivity, sympathovagal balance and sympathovascular transduction.
5. At baseline, individuals with higher baseline office SBP and iLVM had greater sBRS gain, however, patients with higher LVM had impaired cBRS.
6. 50% of the study participants had congenital cerebrovascular abnormalities, consisting of vertebral artery hypoplasia and/or an incomplete circle of Willis, which is higher than the prevalence of these abnormalities in the normotensive population.
7. At baseline, higher relative cerebral blood flow was associated with a higher iLVM in this cohort.
8. There were no correlations between changes in the CBF parameters assessed in this study and changes in office SBP, BRS or sympathovagal balance, 6 months post-RDN.

There was a significant reduction in SBP and LVM in this cohort following RDN, but the mechanism for these effects remain unclear. Since BP reduced and MSNA remained unchanged, a leftward shift in the BRS curve or a reduction in BRS, or increased sympathovascular transduction would have been predicted, but these changes were not observed. This may be due to the small numbers of participants in this pilot study, or confounding effects of variable antihypertensive medication regimes, or the significant

proportion of pre-menopausal women in this study. Even at baseline, there were no correlations between resting levels of the autonomic parameters reported above and baseline office SBP, other than the observation that increased baseline oSBP was associated with increased sBRS, a finding that contradicts established data (Grassi, Seravalle et al. 2014, de Leeuw, Bisognano et al. 2017). Rather confusingly, at baseline, individuals with higher iLVM had greater sBRS gain but impaired cBRS.

The cerebral angiographic findings from this hypertensive cohort were interesting, there was a high prevalence of congenital cerebrovascular abnormalities, which has been previously reported in the hypertensive population, suggesting a potential mechanism driving systemic hypertension to maintain cerebral perfusion (Warnert, Rodrigues et al. 2016). However, at baseline, higher CBF was associated with higher LVM, but not with any increase in MSNA. If higher SBP or MSNA were driving increased CBF, with an adverse effect on left ventricular hypertrophy, we might have hoped to see this relationship in our dataset. Equally, if CBF is preserved, then centrally driven MSNA, and thus SBP, would be expected to reduce and effect positive changes on hypertensive heart disease. Data from larger cohorts, with control subjects, should help to clarify these hypotheses.

Autonomic profiling is feasible in the hypertensive population, but the autonomic mechanisms underpinning any antihypertensive effect of RDN remain to be established.

5.5 Measures of procedural efficacy

5.5.1 Background

5.5.1.1 The need to assess procedural efficacy

Renal denervation remains to be established as a treatment for hypertension. One of the primary concerns following the failure of Symplicity HTN-3 to show a reduction in blood pressure post denervation (Bhatt, Kandzari et al. 2014), has been a method to demonstrate the efficacy of ablation of renal nerves. In Symplicity HTN-3, only 19/364 patients received per-protocol RDN covering all four quadrants of the main renal artery (Bhatt, Kandzari et al. 2014, Kandzari, Bhatt et al. 2015). However, even if 'full coverage' had been achieved, the operator could not have known for certain whether a sufficient proportion of renal nerves had been disrupted, since the procedure is guided by surrogate anatomical markers; a physiological measure would be more robust.

In Symplicity HTN-1 a subset of patients underwent assessment with noradrenaline (NA) spillover, a validated technique for assessing regional sympathetic tone (Meredith, Esler et al. 1991). There was a 47% reduction in NA spillover amongst this cohort, with a BP reduction of 22/12 mmHg, however the authors do not comment on the presence of a correlation between BP response and any reduction in NA spillover (Krum, Schlaich et al. 2009, Esler 2014). Whilst this 47% reduction in renal SNA appeared sufficient to achieve a reduction in BP, this change in SNA is markedly lower than the 70-99% reductions in NA spillover measured across several preclinical studies using surgical and endovascular denervation techniques (Raman, Tsioufis et al. 2017). Further analyses by Esler et al. have shown that denervation following renal nerve ablation is highly variable between individuals and the procedure is clearly far more technically challenging than previously considered (Esler 2014, Tzafriri, Keating et al. 2015).

When the Symplicity catheter was originally launched, operators were advised to prioritise ablation of the proximal superior aspect of the renal artery to target what was believed to be the highest density of renal nerves. However, review of novel anatomical human data indicates that the renal nerves accessible to intraluminal radiofrequency (RF) energy lie more distally in the renal artery adventitia (Sakakura, Ladich et al. 2014); therefore operators following the earlier guidance may have been targeting the wrong part of the artery, resulting in inadequate denervation (Mahfoud, Edelman et al. 2014). More recent guidance therefore advocates ablation in the distal segment of the main renal artery and in larger branch arteries (≥3mm, Symplicity Spyral catheter) where the renal nerves lie closest to the lumen, aiming to reduce the variability in response (Mahfoud, Tunev et al. 2015, Esler and Guo 2017). This approach was employed in the SPYRAL HTN studies (Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018).

Despite advances in catheter technology, complete distal ablation may not always be achievable due to small diameter (<3mm) accessory and branch renal arteries. The Symplicity catheter system monitors tip temperature and impedance during ablation, altering the RF energy delivery in response to a predetermined algorithm. This provides feedback to the operator as to whether a successful ablation has been administered (Krum, Schlaich et al. 2009), but remains a surrogate marker, rather than a measure of

disrupted renal nerve function. The use of alternative denervation modalities, such as targeting high intensity ultrasound or peri-vascular injection of ethanol, may enable disruption of nerves lying further from the vessel lumen, but further validation of these techniques is required, and the issue for confirming successful denervation remains (Fischell, Fischell et al. 2015, Chernin, Szwarcfiter et al. 2017, Raman, Tsioufis et al. 2017).

If the 'completeness' of denervation relates to procedural success, then a method for assessing the degree of renal nerve disruption achieved would be of significant clinical benefit to guide therapy, and additionally guide the development and efficacy of evolving catheter technologies.

5.5.1.2 Existing measures for the peri-procedural quantification of renal denervation

To date, a variety of techniques have been trialled to assess the success of RDN at the time of the procedure. These range from the measurement of biomarkers such as BDNP (brain derived neurotropic factor) (Dörr, Liebetrau et al. 2016) and norepinephrine (Tiroch, Sause et al. 2015), through physiological parameters including renal blood flow (RBF) (Tsioufis, Papademetriou et al. 2013), and direct electrical stimulation of the renal nerves (Chinushi, Izumi et al. 2013, Gal, de Jong et al. 2015, Chinushi, Suzuki et al. 2016, de Jong, Adiyaman et al. 2016, Hoogerwaard, Adiyaman et al. 2018).

Dorr et al. reported a reduction in BDNF measured two hours post RDN, the magnitude of which correlated with the reduction in SBP at 6 months post RDN (Dörr, Liebetrau et al. 2016). A reduction in this sympathetic neuromodulator may provide useful insight into the mechanisms underlying blood pressure reduction following renal nerve ablation, but a sample taken two hours after the procedure, requiring laboratory analysis, does not provide an on-table readout as to whether the operator has administered sufficient ablation therapy to achieve adequate denervation.

Tiroch et al. reported a reduction in the veno-arterial norepinephrine gradient post-RDN in humans (Tiroch, Sause et al. 2015). This reduction was associated with BP responder status three and six months after the procedure, and only those patients with a decrease in veno-arterial NA gradient in both kidneys had a significant reduction in systolic BP. The venous and arterial samples were obtained at the time of the procedure from the renal vessels, but analysed post-hoc, and therefore this method would need to be accelerated in order to provide real-time feedback for the operator to guide denervation.

Tsioufis et al. aimed to address this question by directly assessing renal haemodynamics in swine immediately pre- and post- RDN (Tsioufis, Papademetriou et al. 2013). The group hypothesised that since sympathetic nerve activity causes a reduction in RBF (DiBona 2005), increases in flow would be appreciable after successful denervation. An intra-arterial Doppler flow wire was used to measure the average peak velocity (APV) of blood in the renal artery and additional measures of RBF, renal flow reserve (RFR; the ratio of hyperaemic (dopamine) to basal peak velocity), and renal resistive index (RRI; (peak systolic velocity−end-diastolic velocity)/peak systolic velocity) were calculated. Acutely post-RDN, APV and RBF were increased, and RVR and RRI were reduced, with

changes in these haemodynamic parameters persisting out to 1-month post-RDN. The authors did not state whether the reduction in RBF correlated with a reduction in BP.

An alternative approach has been to focus on the responses of the afferent sensory renal nerves to determine successful ablation. Activation of the renal afferent nerves by ischaemia, hypoxia and intrinsic renal diseases, drives via reflex pathways elevated central sympathetic tone (Biaggioni 1992). Adenosine is released in renal ischaemia and has been shown to activate the afferent renal nerves located in the renal pelvis (Katholi 1983, Katholi, Hageman et al. 1983). In a one kidney, one-clip rat model of hypertension, urinary adenosine concentration was lowered by infusion of adenosine deaminase into the renal artery. When urinary adenosine levels fell, sympathetic nerve activity and hypertension were blunted; this effect was abolished by RDN (Katholi, McCann et al. 1985). In chronically instrumented, uni-nephrectomised sodium-replete conscious dogs, an increase in systemic arterial BP seen in response to intra-renal arterial adenosine infusion (0.6-3 mcg/kg/min) was abolished by renal artery denervation due to the interruption of the renal afferent nerve fibres (Katholi, Whitlow et al. 1984).

Electrical stimulation of the renal nerves is another technique which has been trialled to assess the efficacy of renal denervation. Chinushi et al. demonstrated an increase in systemic blood pressure in response to direct intra-luminal, electrical renal nerve stimulation (RNS) in a canine model, an affect that was substantially attenuated by RDN (Chinushi, Izumi et al. 2013). In contrast, Tsiachris (Tsiachris, Tsioufis et al. 2014) et al. were unable to reproduce these findings in a porcine model. Similar techniques have been used to assess the efficacy of RDN in humans. Pokushalov et al. combined renal denervation with a pulmonary vein isolation ablation procedure for the treatment of atrial fibrillation (Pokushalov, Romanov et al. 2012). They used the same navigation system and catheter as used for the AF ablation to administer high-frequency stimulation to the renal nerves, causing a reflex increase in BP, which was suppressed by renal denervation. Renal denervation was considered to have been achieved when the increase of BP (~15 mmHg from invasive arterial monitoring) was eliminated in the presence of high frequency stimulation. Gal et al. also demonstrated a blunting of the rise in BP in response to electrical RNS following RDN (Gal, de Jong et al. 2015). They used a quadripolar catheter, and following titration of the intensity and duration of the stimulus, renal nerve stimulation (20Hz, 20mA, pulse duration 2ms) was performed at four sites (distal–cranial, distal–caudal, proximal–cranial and proximal–caudal) in both arteries for 1 min. The group also demonstrated a correlation between blunting of the hypertensive response to renal nerve stimulation and the reduction in 24-hour ABPM measures 3-6 months post denervation (de Jong, Adiyaman et al. 2016). The same group now report the ability to differentiate between sympathetic and vagal responses to renal nerve stimulation to guide RDN therapy (de Jong, Hoogerwaard et al. 2018), and the complete blunting of RNS induced temporary rises in augmentation index, pulse pressure, time to maximum systolic pressure and time to reflected wave when considering changes in arterial pressure haemodynamics following RDN (Hoogerwaard, Adiyaman et al. 2018). A major consideration for this approach is the requirement for propofol sedation (Pokushalov, Romanov et al. 2012) or general anaesthesia (Gal, de Jong et al. 2015, de Jong, Adiyaman et al. 2016, de Jong, Hoogerwaard et al. 2018, Hoogerwaard, Adiyaman et al. 2018), to facilitate renal nerve stimulation, which is nociceptive. General anaesthesia has significant safety and logistical implications for a procedure which can otherwise be performed under conscious sedation.

The ideal investigations for assessing the procedural efficacy of RDN would be simple, reproducible, with a clear cut-off to denote adequate ablation, and could be implemented in the setting of the standard catheter laboratory under conscious sedation. The ability to differentiate between afferent and efferent renal nerve denervation would provide additional insight into the mechanisms underpinning RDN as a therapeutic approach for the treatment of resistant hypertension. This study therefore aimed to translate the measures of renal haemodynamic parameters to the acute peri-procedural setting, and differentially assess the function of sympathetic efferent and sensory afferent renal nerves through the quantification of dynamic reflex responses to sympathetic handgrip stress and intra-renal artery adenosine infusion, respectively. We hypothesised that measures which assessed the function of the efferent sympathetic pathway by measuring reflex evoked changes in renal blood flow and renal vascular resistance, and the afferent pathway by looking at reflex changes in blood pressure following activation of the renal chemoreflex by intra-renal artery adenosine infusion, would confirm the procedural success of RDN (see Figure 5-42).

5.5.2 Methods

5.5.2.1 Study participants

Ten patients from the Renal Denervation for Resistant Hypertension pilot study were recruited into this sub-study. Enrolment criteria were the same as for the main study (see Section 5.1.2.2).

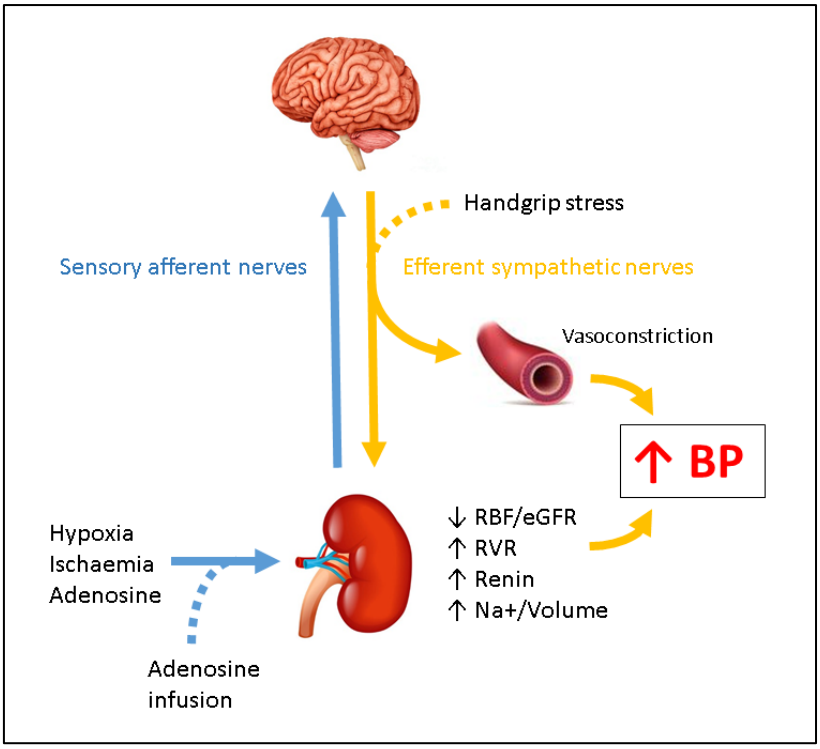


Figure 5-42. Pathways for the assessment of afferent and efferent renal nerve function.

5.5.2.2 Study overview

Physiological monitoring, asepsis and vascular access were obtained according to the standard RDN protocol (Section 4.3.7). The patients received mild intravenous sedation and analgesia (midazolam and fentanyl) prior to ablation and reflex testing, with a view to obtaining a similar level of sedation during physiological testing post denervation (see Figure 5-43 for dosages). The first renal artery (RA) was then cannulated under fluoroscopic guidance and a 0.36 mm Doppler flow wire (ComboMap, Phillips Volcano, USA) was sited in the mid-portion of the main RA lumen and positioned to obtain an optimal signal of flow velocity. Continuous, simultaneous measurements of beat-to-beat non-invasive BP monitoring (Finapres, Finapres Medical Systems, The Netherlands), aortic pressure (Pa), distal pressure at the flow wire tip (Pd), instantaneous peak velocity (IPV) and average peak velocity (APV) were acquired for offline analysis (PowerLab, AD Instruments, Dunedin, New Zealand). BP and APV data were analysed using LabChart (AD Instruments, Dunedin, New Zealand), and IPV data were processed using Spike (Spike 2 v7, Cambridge Electronic Design, Cambridge, UK). For each data acquisition, readings were averaged over 10 seconds and paired with concurrent fluoroscopic images, with measurement of the RA diameter at the tip of the flow wire, to enable calculation of RBF. Volumetric RBF was determined from the relation: RBF (ml/min) = cross-sectional area × (APV (cm/s) × 0.5) x 60, where 0.5 x APV estimates the mean blood flow velocity assuming a time-average parabolic velocity profile across the RA (Doucette, Corl et al. 1992, Savader, Lund et al. 1997). RVR calculated as RVR (mmHg/ml/min) = Pd/RBF. RRI was calculated from the IPV in the renal arteries during the cardiac cycle using the following formula: RI = (peak systolic blood flow velocity−

end-diastolic velocity)/peak systolic blood flow velocity (Savader, Lund et al. 1997, Tsioufis, Papademetriou et al. 2013). Resting haemodynamic parameters, and efferent and afferent nerve reflex responses, were recorded pre-and post-RDN for the first renal artery, and then for the second, contralateral artery. RDN was performed according to the standard study protocol (Section 4.3.7), the protocol for this sub-study is summarised in Box 5.2 below.

5.5.2.3 Assessment of efferent sympathetic renal nerve function

Disruption of efferent renal nerve activity was assessed both by measuring the change in resting renal artery vascular tone following RDN, and by recording the change in RBF, RVR and RRI in response to a sympathetic excitatory stimulus, isometric handgrip (Delaney, Greaney et al. 2010, Jarvis, VanGundy et al. 2011). Prior to arrival in the catheter laboratory, the patient's maximal voluntary contraction (MVC) of the dominant hand was established using 3-5 maximal effort squeezes of the handgrip device. During the procedure, under mild sedation, 2-minute baseline period was recorded followed by handgrip exercise at 40% of the patient's MVC for 90 seconds (Delaney, Greaney et al. 2010). BP, IPV and APV were measured for calculation of RBF, RVR and RRI, at the end of rest and during the last 30 seconds of the handgrip exercise. The test was followed by a 3-minute period of recovery. Patients were encouraged to avoid breath holding and to stay as relaxed as possible during the test even though they were under sedation.

5.5.2.4 Assessment of afferent sensory renal nerve function

The afferent sensory nerves were assessed by recording the systemic BP response to an intra- renal arterial adenosine infusion. Firstly, the dose of adenosine required to achieve a 10-20 mmHg rise in systemic SBP was titrated. A catheter was sited at the ostium of the renal artery and adenosine infused into the artery starting at a rate of 1mcg/kg/min. If a target BP rise of 10-20 mmHg was not seen within 3 minutes, the infusion rate was increased incrementally, in 3-minute stages, to a maximum rate of 140 mcg/kg/min. The initial infusion rate reflects those used in animal studies (Katholi, Hageman et al. 1983, Katholi, Whitlow et al. 1984), and the maximum rate did not exceed 140mcg/kg/min, which is used routinely in the clinical setting for myocardial perfusion studies (Iskandrian 1994). A rise in MAP was reported within 1 minute of commencing intra-renal adenosine infusion in a canine model (Katholi, Hageman et al. 1983). Following a 3-minute washout period, adenosine was then infused at the effective dose identified during the titration for 3 minutes, at the end of which, changes in systemic SBP, along with renal flow haemodynamic parameters, were averaged over a 10 second recording. These measurements were made with the patient in a state of mild conscious sedation.

Catheter Laboratory Protocol

1. Transfer to Catheter Laboratory
2. Mild IV sedation and analgesia
3. Asepsis, femoral arterial puncture, insertion of arterial sheath and anticoagulation with intra-arterial heparin.
4. Cannulation of renal arteries under fluoroscopy guidance including injection of radio-opaque contrast to confirm renal artery anatomy suitable for denervation and to identify anatomical landmarks required for the procedure.
5. Assessment of baseline renal nerve function:
    a. Measurement of change in renal blood flow using intra-arterial flow wire in response to sympathetic stimulus (handgrip exercise).
    b. Adenosine dose titration (first renal artery only).
    c. Localised infusion of adenosine into renal artery and measurement of reflex change in beat-to-beat BP
6. Introduction of ablation catheter via femoral artery under fluoroscopy guidance to distal renal artery, plus further analgesia/sedation.
7. Generator activated; 2 minutes of radiofrequency energy at pre-set level.
8. Repetition of ablation at total of 4-8 discrete sites along renal artery*.
9. Assessment of acute procedural success of denervation:
    a. Measurement of change in renal blood flow using intra-arterial flow wire in response to sympathetic stimulus (handgrip exercise).
    b. Localised infusion of adenosine into renal artery and measurement of reflex change in beat-to-beat BP
10. Contralateral renal artery treated by same method (i.e. repeat steps 5-9)
11. Patient transferred to recovery area and observed closely as sedation wears off. Further analgesia administered if required.

Box 5.2. Catheter laboratory protocol for afferent and efferent renal nerve testing.
*Part way through the study, Medtronic introduced the Symplicity Spyral catheter which has four RF electrodes which can operate simultaneously with a 90 second ablation time.

5.5.3 Results

5.5.3.1 Patient characteristics

Ten patients were recruited to this sub-study. In the first case of the cohort, haemodynamic data were collected using the custom ComboMap software. Unfortunately, it proved difficult to calculate the derived flow and resistance parameters using this software due to limited off-line analysis capabilities. Furthermore, this patient had very tortuous renal artery anatomy and a stable catheter position could not be obtained for the right (second) renal artery. It was not possible to obtain any haemodynamic data from this artery and only one ablation point was administered, making it unlikely that adequate denervation was achieved. For these reasons, the limited data from this patient has not been included in subsequent analyses. The case did highlight data analysis issues, and for all subsequent cases haemodynamic data was recorded via a PowerLab for subsequent off-line analysis.

In another participant, there were technical problems with the flow wire (and a replacement wire) in transducing a Doppler signal, and therefore it was not possible to collect Doppler velocity data for this individual, but BP parameters were recorded per-protocol. I therefore present data for nine patients, with renal artery haemodynamic data present where available (please seen n stated throughout).

Baseline demographic data for the sub-study are shown in Table 5-28. During the RDN procedure itself, the participants received an average of 12 ± 3 ablations in total (six patients treated with the Symplicity Flex catheter, 3 patients treated with the Symplicity Spyral catheter). Over the course of the procedure the patients received, on average, absolute doses of 2.4 ± 0.7 mg of midazolam and 175 ± 30 mcg of fentanyl in analgosedation. The distribution of the drugs at baseline, and then during the first and second renal artery ablations is shown in Figure 5-43. The office and ambulatory BP outcomes are shown in Table 5-29; five of the nine patients responded to RDN with a reduction in office SBP of ≥10 mmHg, however, overall there was no significant reduction in office or 24hr ambulatory BP at either one or six-months post-RDN. The marked variability in the BP response to RDN between individuals is clearly illustrated in Figure 5-44 and consistent with other real-world studies (Kaltenbach, Franke et al. 2013, Bhatt, Kandzari et al. 2014, Persu, Jin et al. 2014).

	BASELINE DATA (N=9)
Age (yrs)	56 ± 12
Male gender	3/9
Antihypertensive medications	5.0 ± 1.9
Office SBP (mmHg)	187 ± 25
Office DBP (mmHg)	101 ± 22
24hr SBP (mmHg)	169 ± 10
24hr DBP (mmHg)	94 ± 13

Table 5-28. Patient demographics for afferent and efferent renal nerve testing sub-study.
SBP; systolic blood pressure, DBP; diastolic blood pressure. Mean ± SD.

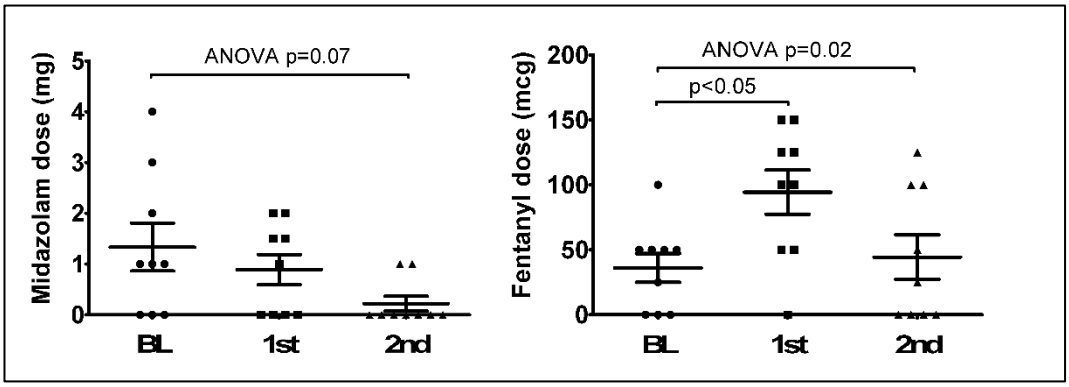


Figure 5-43. Doses of midazolam and fentanyl given at baseline (BL) and during the 1st and 2nd, contralateral, renal denervation.

CHANGE IN BP	1 MONTH			6 MONTHS		
		N	P		N	P
Office SBP (mmHg)	-5.3 ± 12.5	9	0.68	2.1 ± 12.4	8	0.87
Office DBP (mmHg)	-0.4 ± 5.6	9	0.95	2.9 ± 6.0	8	0.64
24hr SBP (mmHg)	-2.3 ± 4.4	6	0.62	-4.4 ± 6.7	5	0.55
24hr DBP (mmHg)	0.0 ± 2.8	6	1.0	1.0 ± 4.5	5	0.84

Table 5-29. Change in blood pressure following renal denervation for afferent and efferent renal nerve testing sub-study.
SBP; systolic blood pressure, DBP; diastolic blood pressure.

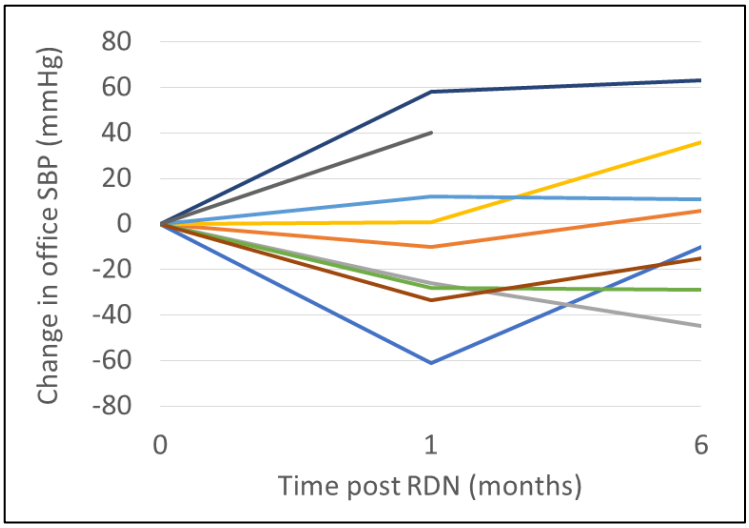


Figure 5-44. Change in office systolic blood pressure (SBP) following renal denervation (RDN) for afferent and efferent renal nerve testing sub-study.

There was a significant correlation between the number of ablations received by a patient (total number of ablations for both renal arteries, R=0.77, p=0.01). Interestingly, in this cohort the correlation is the inverse of patterns previously reported, with those with the fewest ablations responding to RDN (Figure 5-45).

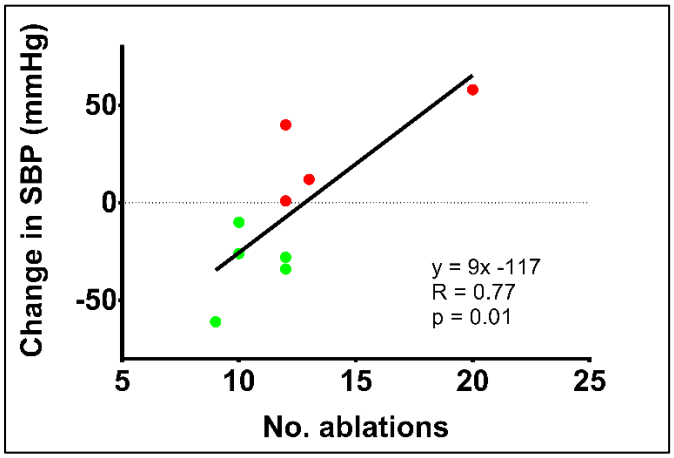


Figure 5-45. Correlation between number of ablation points and the change in SBP at 1-month post denervation.
SBP; systolic blood pressure. Responders in green, non-responders in red.

5.5.3.2 Resting haemodynamic parameters

The resting haemodynamic data pre- and post-RDN are summarised in Table 5-30. There was a significant increase in APV in the first renal artery treated with RDN (p=0.047), but this was not seen in the second vessel and was not associated with an increase in RBF in either artery. There was a significant difference between the change in APV post-RDN between the first and second renal arteries (18.7 ± 7.8 vs 0.7 ±2.6 cm/s, p=0.045), primarily reflecting the difference between the change in diastolic velocities, but this was the only difference in the outcomes measured between the first and second arteries. There was no difference in any parameter between the first measures taken prior to RDN in the first renal artery, and the final measurements taken post RDN in the second renal artery (although please note the statistics presented are for a paired t-test, and therefore only include those patients with data available at both specified timepoints). When the data from both renal arteries were pooled, they showed a borderline increase in APV post RDN (9.7 ± 5.6 cm/s, p=0.05), however no change in resting RBF or RVR was seen for either artery (Figure 5-46).

The RBF data in Figure 5-46 show an outlier with a very high blood flow. This patient had ectatic renal arteries, and in this artery the proximal vessel was narrow, dilating up in the more distal portion. The tip of the flow wire (point at which RA diameter measured) was sited just into the wider portion of the artery, and the larger CSA at this point, along

with the high velocity persisting from the narrow portion of the vessel, may well explain the extremely high RBF calculated.

Five of the nine patients had responded to RDN with a reduction in office SBP of ≥10 mmHg at 1-month post RDN. Amongst these responders there was no significant change in any of the resting renal artery haemodynamic parameters pre- versus post-RDN. Interestingly, when looking at the pooled data for both renal arteries, the non-responders showed a borderline increase in APV post RDN (16 ± 8 cm/s, n=8, p=0.08), however there was no significant difference in the change in any haemodynamic parameter between responders and non-responders following RDN.

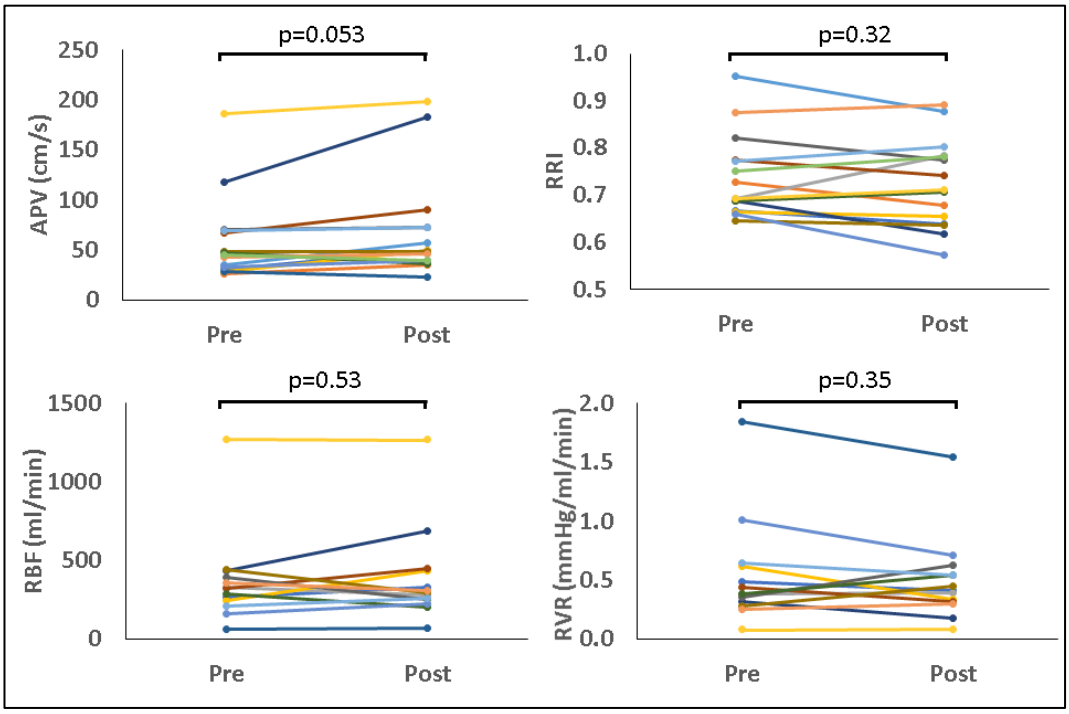


Figure 5-46. Resting haemodynamic parameters before and after RDN.
The changes in resting average peak velocity (APV), renal resistive index (RRI), renal blood flow (RBF) and renal vascular resistance (RVR) before versus after renal denervation (RDN) for the individual study participants.

	1st Renal Artery				2nd Renal Artery				Bilateral Renal Arteries				Start vs end	
	Pre-RDN	Post- RDN	N	P	Pre-RDN	Post- RDN	N	P	Pre-RDN	Post- RDN	N	P	N	p
SBP (mmHg)	188 ± 8.1	184 ± 10	9	0.48	181 ± 9	181 ± 10	9	0.97	184 ± 6	182 ± 7	18	0.50	9	0.30
DBP (mmHg)	88 ± 6.6	87 ± 7	9	0.65	86 ± 7	87 ± 6.9	9	0.56	87 ± 5	87 ± 5	18	0.90	9	0.64
MAP (mmHg)	125 ± 6.6	123 ± 7	9	0.66	122 ± 7	122 ± 7	9	0.78	123 ± 5	123 ± 5	18	0.76	9	0.51
HR (bpm)	65 ± 2.6	65 ± 3	9	0.97	66 ± 3	65 ± 3	8	0.42	66 ± 2	65 ± 2	17	0.63	8	0.90
APV (cm/s)	53 ± 11	72 ± 17	8	0.047	62 ± 18	63 ± 20	8	0.81	58 ± 10	67 ± 13	16	0.05	8	0.41
RA diam (cm)	0.51 ± 0.03	0.49 ± 0.04	6	0.35	0.48 ± 0.05	0.47 ± 0.03	7	0.51	0.50 ± 0.03	0.48 ± 0.02	13	0.24	5	0.40
RBF (ml/min)	330 ± 30	405 ± 65	6	0.27	397 ± 153	373 ± 152	7	0.43	366 ± 81	388 ± 84	13	0.53	5	0.51
RVR (mmHg/ml/min)	0.43 ± 0.04	0.37 ± 0.06	6	0.49	0.64 ± 0.23	0.59 ± 0.17	7	0.57	0.54 ± 0.13	0.49 ± 0.10	13	0.35	5	0.81
Peak systolic velocity (cm/s)	114 ± 19	137 ± 26	8	0.14	133 ± 33	142 ± 37	7	0.12	123 ± 18	139 ± 21	15	0.0496	7	0.53
End diastolic velocity (cm/s)	27 ± 6	38 ± 11	8	0.11	36 ± 11	37 ± 11	7	0.51	31 ± 6	38 ± 7	15	0.09	7	0.26
RRI	0.75 ± 0.03	0.72 ± 0.03	8	0.20	0.73 ± 0.03	0.73 ± 0.04	7	0.85	0.74 ± 0.02	0.72 ± 0.02	15	0.32	7	0.25

Table 5-30. Resting haemodynamic data. Data reported before and after RDN for the first and second renal arteries treated, the pooled data for both renal arteries, and the comparison between the measurements taken before RDN in the first renal artery and after RDN in the second renal artery at the end of the procedure. SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, HR; heart rate, APV; average peak velocity, RA diam; renal artery diameter, RBF; real blood flow, RVR; renal vascular resistance, RRI; renal resistive index.

	PRE RDN				POST RDN				ΔΔ	
Handgrip	Rest	Stress	N	P	Rest	Stress	N	p	N	p
SBP (mmHg)	185 ± 6	217 ± 9	16	0.0002	181 ± 8	212 ± 8	15	0.001	15	0.77
DBP (mmHg)	87 ± 5	106 ± 5	16	0.0006	85 ± 6	104 ± 5	15	0.003	15	0.86
MAP (mmHg)	123 ± 5	150 ± 6	16	0.0004	121 ± 6	147 ± 5	15	0.001	15	0.87
HR (bpm)	66 ± 2	75 ± 2	16	0.004	63 ± 2	73 ± 2	15	0.002	15	0.97
APV (cm/s)	58 ± 10	56 ± 8	16	0.70	69 ± 14	73 ± 13	15	0.29	15	0.25
RA diam (cm)	0.50 ± 0.03	0.51 ± 0.03	15	0.46	0.47 ± 0.02	0.49 ± 0.03	13	0.17	12	0.78
RBF (ml/min)	346 ± 72	345 ± 51	15	0.96	380 ± 85	449 ± 90	13	0.11	12	0.12
RVR (mmHg/ml/min)	0.59 ± 0.13	0.60 ± 0.10	15	0.60	0.52 ± 0.10	0.56 ± 0.15	13	0.43	12	0.90
Peak systolic velocity (cm/s)	123 ± 18	117 ± 16	15	0.18	141 ± 24	145 ± 24	13	0.46	13	0.22
End diastolic velocity (cm/s)	31 ± 6	27 ± 4	15	0.13	38 ± 9	41 ± 8	13	0.48	13	0.41
RRI	0.74 ± 0.02	0.75 ± 0.02	15	0.48	0.73 ± 0.03	0.72 ± 0.03	13	0.51	13	0.29

Table 5-31. Isometric hand grip data.

Bilateral data showing the change in renal haemodynamic parameters with isometric handgrip. The final column shows the level of significance for the difference between each parameter pre- and post-RDN. SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, HR; heart rate, APV; average peak velocity, RA diam; renal artery diameter, RBF; real blood flow, RVR; renal vascular resistance, RRI; renal resistive index. Mean ± SEM.

5.5.3.3 Dynamic efferent renal nerve testing

5.5.3.3.1 Isometric handgrip

Participants were able to perform isometric handgrip exercise as a sympathetic stimulus at all stages of the procedure; MAP was significantly increased from rest by handgrip both before and after RDN, with no difference between the change in MAP before and after RDN (see Table 5-31). This was also the case when the data were analysed for both the first and second renal arteries (Δ: pre-RDN 22 ± 6 mmHg (p=0.02) and post-RDN 23 ± 11 mmHg (p=0.04); pre-RDN 31 ± 10 mmHg (p=0.01) and post-RDN 30 ± 5 mmHg (p=0.01), respectively). There was no significant difference on handgrip testing between the first and second renal artery for any of the haemodynamic parameters; the following analysis therefore describes the pooled data for both arteries.

5.5.3.3.2 Full cohort data

When considering the whole cohort, there were no significant changes in the absolute values of any of the flow or resistance parameters, before or after RDN, despite the increased BP with handgrip stress (see Table 5-31). Furthermore, the changes in these parameters did not differ pre- versus post-RDN (see Table 5-31). Given the wide range in the resting levels of RBF (59 - 1269 ml/min), data were analysed using percent change in the haemodynamic parameters, and results compared for RDN responders (≥10 mmHg reduction in office SBP at 1-month post RDN) and non-responders.

Over the whole cohort, pooling data from both renal arteries, there was a significant percentage increase in RVR in response to handgrip pre-RDN, not seen post procedure, although the difference between these changes was not significant (18 ± 7%, p=0.02; 5 ± 7%, p=0.49, respectively; Δp=0.20, see Table 5-32). Percentage changes in the other haemodynamic parameters did not differ before and after RDN (see Table 5-32).

	PRE-RDN			**POST-RDN**			**Δ**	
Handgrip	% Change	N	P	% Change	N	P	N	P
SBP (mmHg)	17 ± 4	16	0.0002	18 ± 5	15	0.002	15	0.93
DBP (mmHg)	28 ± 9	16	0.005	28 ± 9	15	0.007	15	0.93
MAP (mmHg)	24 ± 6	16	0.0009	25 ± 7	15	0.002	15	0.94
HR (bpm)	16 ± 5	16	0.006	16 ± 4	15	0.002	15	0.96
APV (cm/s)	3 ± 5	16	0.53	13 ± 11	15	0.26	15	0.45
RA diam (cm)	3 ± 3	15	0.30	4 ± 3	13	0.18	12	0.95
RBF (ml/min)	11 ± 7	15	0.17	24 ± 14	13	0.12	12	0.24

RVR (mmHg/ml/min)	18 ± 7	15	0.02	5 ± 7	13	0.49	12	0.17
Peak systolic velocity (cm/s)	-4 ± 3	15	0.20	6 ± 6	13	0.38	13	0.25
End diastolic velocity (cm/s)	-2 ± 11	15	0.57	14 ± 15	13	0.36	13	0.43
RRI	2 ± 2	15	0.49	-1 ± 2	13	0.55	13	0.28

Table 5-32. Percentage change in haemodynamic parameters measured in response to a sympathetic stressor - handgrip.
The percentage change in systemic BP and renal haemodynamics in response to handgrip stress, and the level of significance for the difference in these percentage changes pre- versus post-RDN (final column). SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, HR; heart rate, APV; average peak velocity, RA diam; renal artery diameter, RBF; real blood flow, RVR; renal vascular resistance, RRI; renal resistive index. Mean ± SEM.

5.5.3.3.3 Correlations between response to handgrip and blood pressure outcomes

There was a significant correlation between the percentage change in RVR with handgrip post-RDN and the change in office SBP at 1 month (R=0.6, p=0.03), and a trend towards a correlation between the percentage change in RBF with handgrip post-RDN and the change in SBP at 1 month (see Figure 5-47). Patients responding to RDN tended to have an increase in RBF and a decrease in RVR in response to sympathetic stress following renal nerve ablation.

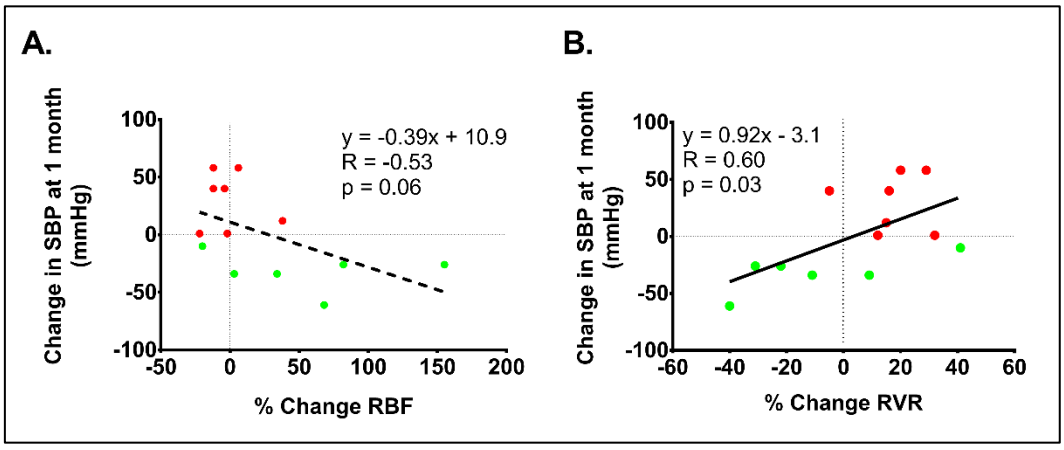


Figure 5-47. Correlations between the BP response to RDN at 1 month, and the changes in renal haemodynamics with sympathetic stress post-RDN.
Correlations between the percentage change in A. renal blood flow (RBF) and B. renal vascular resistance (RVR) in response to handgrip stress following renal denervation (RDN). Responders shown in green and non-responders in red.

5.5.3.3.4 Responders versus non-responders

The data were analysed by BP outcome. Prior to denervation, there was no difference in the percentage change in renal haemodynamic response to handgrip between responders (n=5) and non-responders (n=4); RBF: 21 ± 11% vs -1 ± 8% (p=0.38), RVR: 8 ± 9% vs 29 ± 11% (p=0.20), respectively. Following RDN, the differences in haemodynamic parameters between responders and non-responders were borderline significant; RBF: 54 ± 26% vs -1 ± 7%, RVR: -9 ± 12% vs 17 ± 5%, respectively (both p=0.05, see Figure 5-48).

Post-RDN, the responders had a trend towards a percentage increase in RBF with handgrip (54 ± 26%, p=0.09), although this did not differ significantly from the pre-RDN level (21 ± 11%, p=0.1; Δ p=0.22). In comparison, the non-responders had absolutely no difference in the percentage change in RBF pre- versus post-RDN, with no change in RBF with handgrip either before or after the procedure (Δ p=0.96; pre: -1 ± 8%, p=0.88; post: -1 ± 7%, p=0.88). The non-responders, who may have intact renal nerves, maintained stable RBF before and after RDN despite an increase in perfusion pressure (increased MAP with handgrip) with a significant increase in RVR (change in RVR; pre: 29 ± 11%, p=0.03; post: 17 ± 5%, p=0.01; Δ p=0.30). For the RDN-responders, the pattern of data shows a reduction, rather than increase, in RVR with handgrip post-RDN, albeit non-significant (-9 ± 12%, p=0.49). Of note, the rise in RVR with handgrip pre-RDN in the responder group fails to reach significance (8 ± 9%, p=0.39) and does not differ from the fall in RVR seen post ablation (Δ p=0.26). There were no significant differences in RRI, within or between outcome groups, before or after RDN.

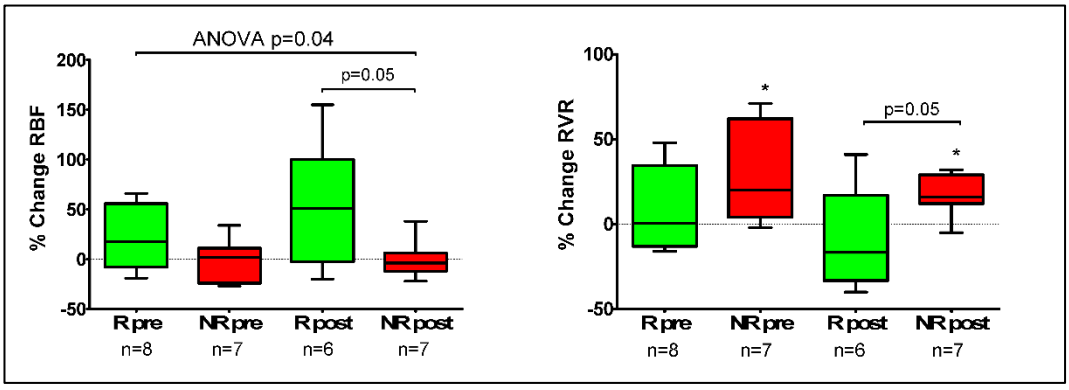


Figure 5-48. Percentage change in renal blood flow and renal vascular resistance in response to handgrip stress, in RDN responders and non-responders.
Pooled data from both arteries, presented for responders (R, green) and non-responders (NR, red) both pre-and post-RDN. RBF; renal blood flow, RVR; renal vascular resistance. *p<0.05.

5.5.3.4 Dynamic afferent renal nerve testing

5.5.3.4.1 Adenosine dose titration

Afferent renal nerve integrity was assessed using an infusion of adenosine directly into the main renal artery, with measurement of the reflex change in systemic blood pressure. This technique has not been trialled in humans, and therefore the first stage

was to complete an adenosine dose titration. The results of the titration are shown in Figure 5-49. The dose range required to achieve a ≥10 mmHg rise in systemic SBP was 10-50 mcg/kg/min (median 30 mcg/kg/min), although three participants reached the maximum dose of 140 mcg/kg/min without a rise in SBP, with BP falling in these individuals at higher doses. There was no significant correlation between the peak dose of the adenosine titration and the change in SBP at 1-month post RDN (R=0.40, p=0.28).

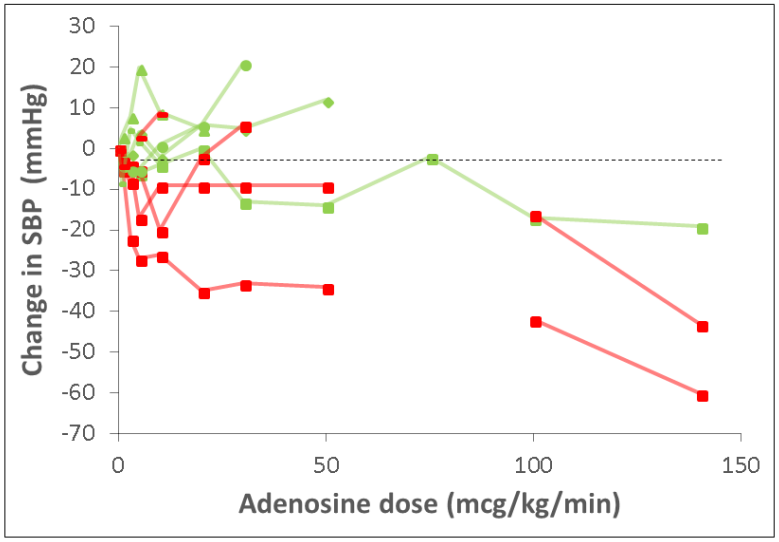


Figure 5-49. Adenosine dose titration.
Change in systemic systolic blood pressure (SBP) in response to three-minute increments in adenosine dose, up to a target SBP rise of ≥10 mmHg. RDN BP responders are shown in green, and non-responders in red.

The study was designed to look for reproducibility in the response to adenosine to ensure that there was no tolerance to the stimulus. Therefore, after the initial dose titration there was a 3-minute washout period and then the titrated dose of adenosine was infused again, for a further 3 minutes, to confirm the SBP response. The average change in systemic SBP at the peak adenosine dose during titration was -5 ± 7 mmHg, despite the titration targeting a ≥10 mmHg rise in SBP due to the variable BP response to adenosine, including a reduction in SBP in some cases (see Figure 5-49). The change in SBP at the peak titration dose was significantly different to the SBP response to adenosine measured prior to RDN in the first renal artery, with a rise in SBP on repeat infusion of adenosine after the washout period (8 ± 6 mmHg, p=0.04).

5.5.3.4.2 Full cohort data

The absolute values for the BP and renal haemodynamic parameters, pooled for both first and second renal arteries, pre- and post-RDN, are shown in Table 5-33.

5.5.3.4.2.1 Blood pressure response to adenosine infusion:

There was no significant change in systemic BP with infusion of adenosine prior to RDN for the bilateral data. Post-RDN, there was a significant reduction in SBP (-9 ± 3 mmHg,

p=0.01) in response to adenosine infusion, which was significantly different from the baseline response (p=0.03, Table 5-33).

To address the variation baseline blood pressure levels, the pooled data from both arteries were analysed as percent change in each of the parameters in response to a 3-minute adenosine infusion into the renal artery immediately pre-, versus immediately post-RDN (see Table 5-34). As with the absolute numerical data, there was a reduction in BP response to adenosine post-RDN, not seen prior to the procedure.

5.5.3.4.2.2 Renal haemodynamic response to adenosine infusion:

Looking at the absolute numerical data pooled for both renal arteries, there were no significant changes in any renal haemodynamic parameter with infusion of adenosine prior to RDN (Table 5-33). Post-RDN, there was a trend towards a difference in the change in RBF pre- versus post-RDN (p=0.09). RBF did not change in response to adenosine infusion pre-RDN (-3 ± 32 ml/min, n=12, p=0.93), therefore this difference is likely attributable to a possible increase in RBF post-RDN (+68 ± 57 ml/min, n=10, p=0.27). Although the signal is weak, the data suggest the converse relationship for RVR; there was a potential increase in RVR with adenosine pre-RDN (+0.08 ± 0.07 mmHg/ml/min, n=12, p=0.28), but no change in RVR in response to adenosine post RDN (-0.01 ± 0.04 mmHg/ml/min, n=10, p=0.81; Δ pre vs Δ post p=0.12 (Table 5-33)).

Considering the percent change data, there was a trend towards a difference between the percentage change in RBF in response to adenosine pre- versus post-RDN (p=0.06), with a minimal fall in RBF with adenosine pre-RDN and an increase in RBF with this afferent stimulus post RDN (see Table 5-34). There was a significant difference between the percentage change in RVR in response to adenosine before and after RDN (p=0.02); RVR increased in response to adenosine pre-RDN but had an opposite response after ablation, however, these changes did not attain significance (see Table 5-34).

	PRE-RDN				POST-RDN				ΔΔ	
	Rest	Adenosine	N	P	Rest	Adenosine	N	P	N	p
SBP (mmHg)	187 ± 6	188 ± 7	18	0.78	185 ± 7	177 ± 7	18	0.01	18	0.03
DBP (mmHg)	87 ± 5	88 ± 6	18	0.69	87 ± 4	82 ± 6	18	0.01	18	0.009
MAP (mmHg)	123 ± 5	126 ± 5	18	0.43	124 ± 4	118 ± 6	18	0.02	18	0.02
HR (bpm)	67 ± 1	69 ± 3	18	0.22	66 ± 2	71 ± 3	18	0.06	18	0.12
APV (cm/s)	70 ± 14	75 ± 18	15	0.52	67 ± 13	76 ± 19	15	0.20	15	0.58
RA diam (cm)	0.52 ± 0.03	0.53 ± 0.03	13	0.75	0.51 ± 0.03	0.51 ± 0.03	10	0.91	9	0.36
RBF (ml/min)	439 ± 70	436 ± 79	12	0.93	498 ± 111	565 ± 160	10	0.27	9	0.09
RVR (mmHg/ml/min)	0.39 ± 0.07	0.47 ± 0.13	12	0.28	0.38 ± 0.08	0.37 ± 0.09	10	0.81	9	0.12
Peak systolic velocity (cm/s)	147 ± 25	162 ± 28	13	0.23	142 ± 24	161 ± 36	14	0.20	12	0.59
End diastolic velocity (cm/s)	42 ± 13	60 ± 19	13	0.23	36 ± 9	48 ± 18	14	0.29	12	0.70
RRI	0.73 ± 0.03	0.68 ± 0.07	13	0.46	0.74 ± 0.02	0.76 ± 0.03	14	0.60	12	0.26

Table 5-33. Haemodynamic response to adenosine infusion at the peak titrated dose.

Bilateral data showing the absolute data for systemic blood pressure and renal haemodynamic parameters before and after renal denervation in response to a 3-minute adenosine infusion at the peak titrated dose. The final column shows the level of significance for the difference between each parameter pre- and post-RDN. SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, HR; heart rate, APV; average peak velocity, RA diam; renal artery diameter, RBF; real blood flow, RVR; renal vascular resistance, RRI; renal resistive index. Mean ± SEM.

	PRE-RDN			POST-RDN			Δ	
Adenosine	% Change	N	P	% Change	N	P	N	P
SBP (mmHg)	0.6 ± 1.9	18	0.75	-4.5 ± 1.7	18	0.02	18	0.03
DBP (mmHg)	0.04 ± 2.2	18	0.99	-7.0 ± 2.5	18	0.01	18	0.008
MAP (mmHg)	2.9 ± 3.5	18	0.41	-5.3 ± 2.0	18	0.02	18	0.04
HR (bpm)	3.5 ± 2.9	18	0.24	8.2 ± 4.1	18	0.06	18	0.11
APV (cm/s)	3.6 ± 8.3	15	0.67	3.3 ± 6.5	15	0.63	14	0.94
RA diam (cm)	1.0 ± 3.2	13	0.77	-0.03 ± 1.8	10	0.99	9	0.42
RBF (ml/min)	-1.5 ± 9.0	12	0.87	6.5 ± 8.0	10	0.44	9	0.06
RVR (mmHg/ml/min)	11.8 ± 10.9	12	0.30	-8.5 ± 7.5	10	0.29	9	0.02
Peak systolic velocity (cm/s)	11.5 ± 9.0	13	0.23	6.2 ± 6.8	14	0.39	12	0.75
End diastolic velocity (cm/s)	40.4 ± 33.0	13	0.24	8.3 ± 15.6	14	0.60	12	0.30
RRI	-5.9 ± 8.5	13	0.50	2.9 ± 4.4	14	0.52	12	0.25

Table 5-34. Percentage change in systemic blood pressure and renal haemodynamics in response to renal arterial adenosine infusion as an afferent stimulus.
SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, HR; heart rate, APV; average peak velocity, RA diam; renal artery diameter, RBF; real blood flow, RVR; renal vascular resistance, RRI; renal resistive index. Mean ± SEM.

5.5.3.4.3 Correlations between adenosine response and blood pressure outcomes

The bilateral pooled-data for percentage change in response to adenosine summarised in Table 5-34 have been correlated against the blood pressure response to RDN at 1 month. Pre-RDN, the only parameter that correlated with blood pressure response to RDN was the percentage change in renal artery diameter in response to adenosine infusion; RDN responders tended to increase RA diameter (R=-0.6, p=0.03, see Figure 5-50). Post-RDN, there was a significant negative correlation between percentage change in DBP in response to adenosine and the medium term SBP response, with trends seen for the changes in SBP, RBF and RVR (see Figure 5-51).

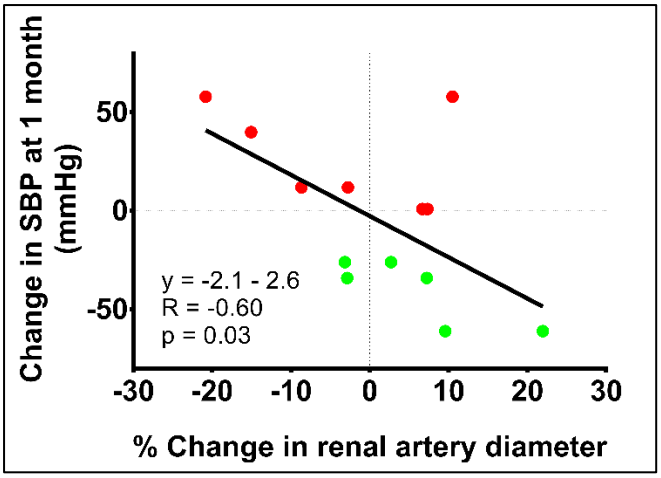


Figure 5-50. Correlation between the percentage change in renal artery diameter in response to adenosine infusion pre-RDN, and the office systolic blood pressure outcome at 1 month.
Responders shown in green and non-responders in red.

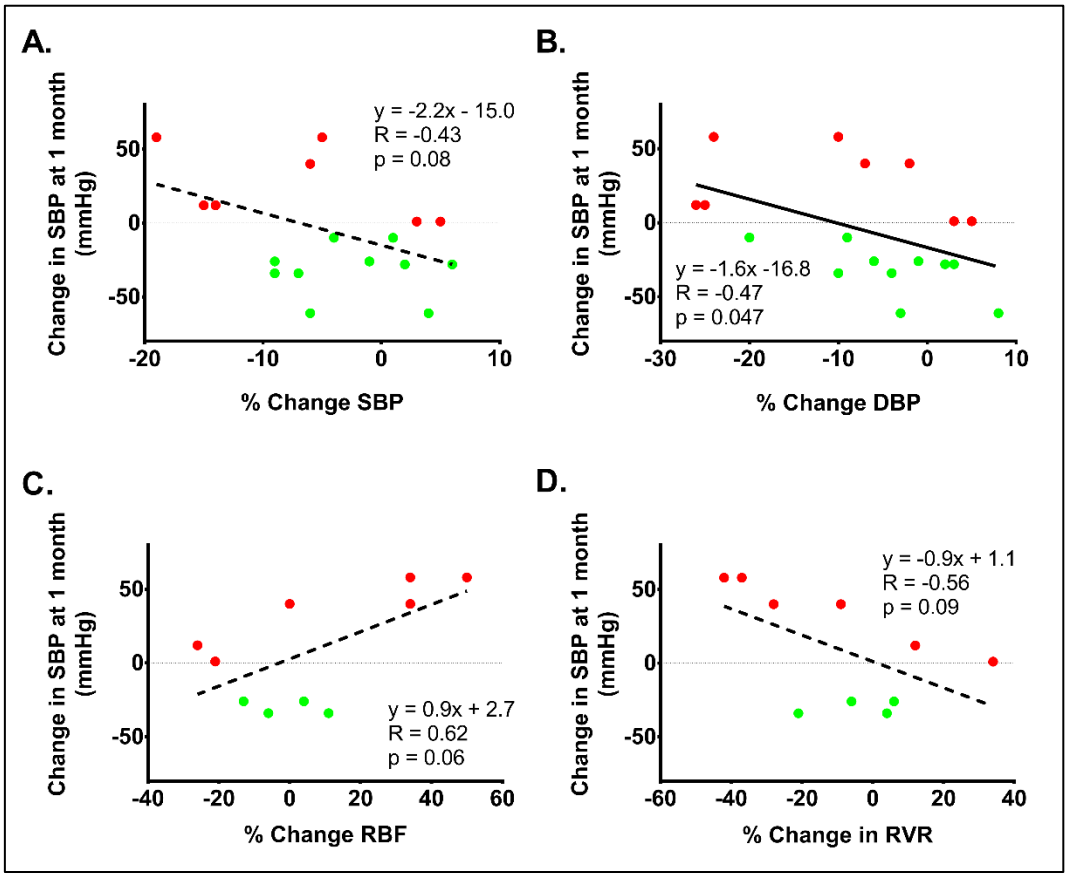


Figure 5-51. Correlations between the change in office SBP at 1-month post-RDN, and the percentage changes in A. systolic blood pressure, B. diastolic blood pressure, C. renal blood flow and D. renal vascular resistance in response to renal arterial adenosine at time of procedure, post-RDN.
SBP; systolic blood pressure, DBP: diastolic blood pressure, RBF; renal blood flow, RVR; renal vascular resistance. Responders shown in green and non-responders in red.

5.5.3.4.4 First versus second renal artery ablated

The systemic SBP response to adenosine did differ between ablation of the first and second renal arteries. In the first renal artery treated (left in seven patients, right in two patients), there was a non-significant rise in SBP with adenosine observed pre- RDN (8 ± 6 mmHg, p=0.19). This was significantly different (p<0.05) from the fall in SBP post-RDN in the first renal artery (-16 ± 6 mmHg, p=0.02, see Figure 5-52), and could be consistent with ablation of the afferent nerves and disruption of the reflex increase in systemic BP in response to intra-renal arterial adenosine. However, there was a fall in SBP in response to adenosine seen pre-RDN in the second renal artery (-6 ± 2 mmHg, p=0.02), with no change in SBP with adenosine post-RDN in this artery (-3 ± 5 mmHg, p=0.51). There were no differences between any of the renal blood flow or resistance parameters between the first and second renal arteries at any stage of the afferent nerve testing study. There was no evidence of sidedness, with no difference between the blood pressure or renal haemodynamic parameters in response to adenosine between the right and left renal arteries.

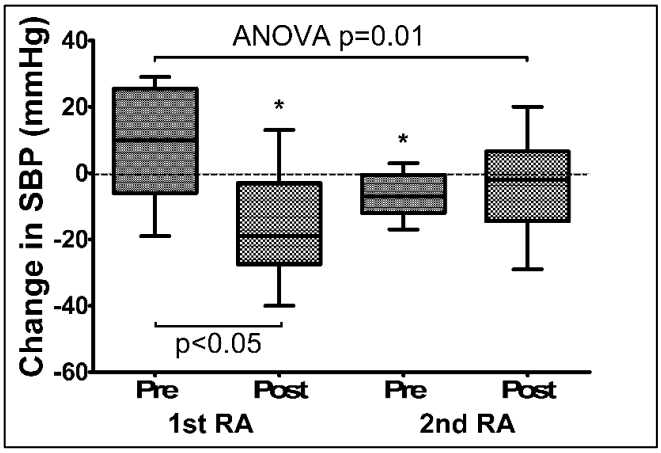


Figure 5-52. Reflex change in systolic blood pressure in response to renal arterial adenosine infusion, pre- and post-RDN, for the first and second renal arteries (RA) ablated.
All groups n=9. *One sample T-test vs zero change, p=0.02

5.5.3.4.5 Responders versus non-responders

The data for percentage change in the haemodynamic parameters recorded in response to adenosine infusion have been further analysed by RDN BP outcome (see Figure 5-53). Amongst RDN responders, there were no significant percentage changes in any variable in response to adenosine either pre- or post-ablation, and no difference in the percentage change in the haemodynamic parameters before versus after RDN.

Amongst the non-responders, there were no significant percentage changes in any parameter pre-RDN, but there was a significant percentage reduction in SBP post-RDN (-7 ± 3 %, p=0.048). Comparing the data pre- versus post-RDN, there was a significant difference between the percentage changes pre-and post-RDN in non-responders with data available at both time-points (note low n), for SBP (n=8, p=0.01) and RBF (p=0.048, n=5), with a borderline difference for RVR (p=0.07, n=5) (see Figure 5-53). However,

there was no difference between the percentage changes in any parameter, either pre- or post-RDN, between responders and non-responders to adenosine.

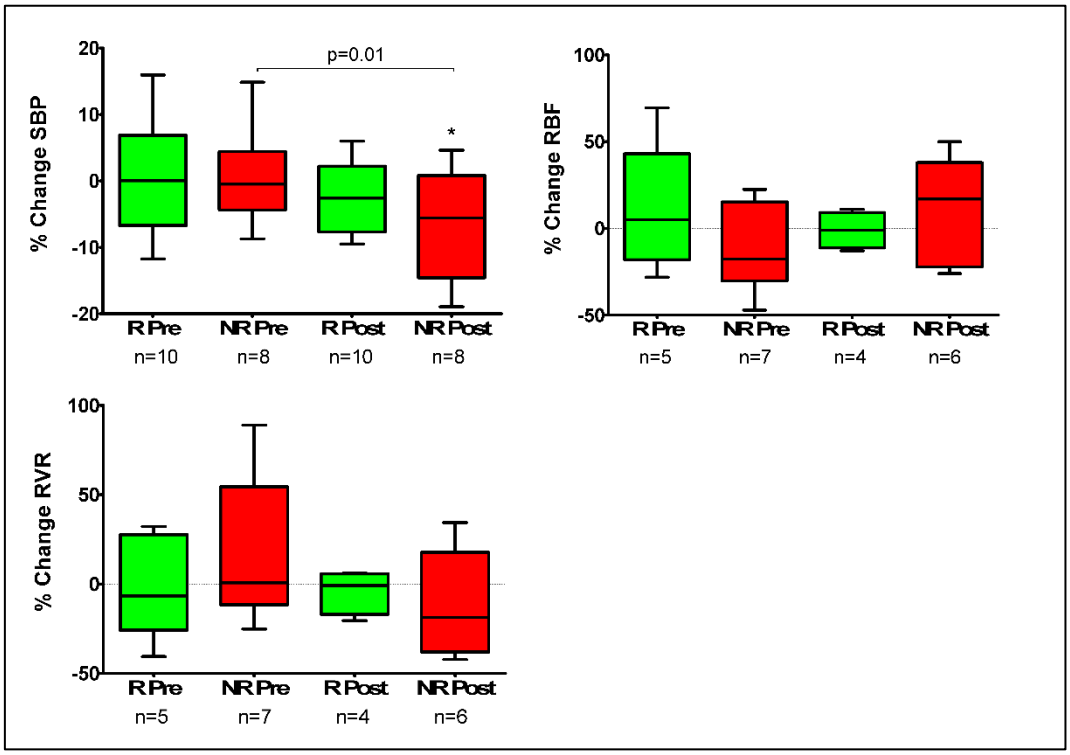


Figure 5-53. Percentage change in systemic systolic blood pressure, renal blood flow and renal vascular resistance in response to renal arterial adenosine infusion, by SBP response to renal denervation.

Pooled data from both arteries, presented for responders (R) and non-responders (NR) both pre-and post-RDN. SBP; systolic blood pressure, RBF; renal blood flow, RVR; renal vascular resistance. *p<0.05. Responders shown in green and non-responders in red.

5.5.4 Discussion

This sub-study aimed to find an effective test to establish adequate renal nerve ablation at the time of an RDN procedure. This strategy hoped to give a real-time measure that could guide the operator and be used in the standard catheter laboratory setting, under conscious sedation. The use of a dynamic sympathetic stressor in this study shows promise as an investigative tool, with an inability to increase RVR with handgrip post-RDN indicating disruption of the sympathetic vascular reflex, but this technique may lack sensitivity to guide ablation in the individual patient.

In this study cohort a greater number of ablation points was associated with a failure to respond to denervation, and whilst this contradicts some (Kandzari, Bhatt et al. 2015, Burchell, Chan et al. 2016), but not all (Vogel, Kirchberger et al. 2014, Sharp, Davies et al. 2016), published data, it does emphasise the importance of a physiological, rather than anatomical, measure of procedural efficacy.

In humans, changes in renal haemodynamics have been assessed non-invasively during long-term follow-up after RDN. Mahfoud et al. report a reduction in RRI measured using transcutaneous duplex ultrasound at 3 and 6 months post RDN, however, the reduction in RRI did not correlate with the reduction in BP reported (Mahfoud, Cremers et al. 2012). RRI is a marker of intraparenchymal resistance and may provide information about subclinical atherosclerosis, as well as correlating with target organ damage in hypertension (Pontremoli, Viazzi et al. 1999, Viazzi, Leoncini et al. 2014). An improvement in RRI may therefore have clinical relevance. Data from Ott et al. using magnetic resonance perfusion imaging provide further insight; RVR was reduced at 3 months post-RDN, but renal perfusion remained unchanged despite a reduction in the perfusing pressure (SBP), indicating preserved autoregulation (Ott, Janka et al. 2013). Similarly, using MRI, Doltra et al. reported an increase in renal artery cross-sectional area, peak velocity and blood flow at 6 months post-RDN, which did not differ between BP responders and non-responders (Doltra, Hartmann et al. 2016). These data support the concept that RDN can increase RBF and reduce RVR through disruption of the efferent sympathetic nerves, but do not support the use of renal haemodynamic parameters as markers for either patient selection, or procedural efficacy in the context of RDN, and do not provide peri-procedural feedback for the operator since they are based on data from long-term follow-up.

This study investigated the acute changes in resting renal haemodynamic parameters measured at the time of the procedure but was unable to replicate the increase in resting RBF post RDN reported in swine by Tsioufis et al. (Tsioufis, Papademetriou et al. 2013). This is interesting, as one would expect a reduction in RVR following renal artery denervation if the vessel was subject to resting sympathetic tone (Dibona 2000). The lack of change in resting parameters in this cohort may be due to inadequate denervation, although this would require the blood pressure reductions in our responder group to be attributed to vascular resistance reduction in other beds. It is possible that when the renal nerves are disrupted, local autoregulatory pathways can maintain steady-state renal blood flow under resting conditions. Given the kidney's ability to autoregulate effectively, the addition of dynamic physiological stressors to

challenge the integrity of the efferent and afferent renal nerves may prove more robust and enable the 'signal' to rise above the noise.

5.5.4.1 Efferent renal nerve responses to handgrip stress

Our data demonstrate that handgrip stress was both physically possible, and physiologically able, to act as a sympathetic stressor (significantly increasing systemic BP and heart rate, see Table 5-31) throughout the RDN procedure, in patients under conscious sedation. Looking at the bilateral renal artery data for all patients, there was an increase in RVR in response to handgrip stress prior to RDN; renal blood flow was maintained in the face of a higher perfusion pressure. This response was not seen when averaging the cohort data after denervation. Looking at the results by BP outcome, both RDN responders and non-responders increased RVR and maintained RBF in response to handgrip stress pre-RDN (although the rise in RVR did not reach significance for the responder group, Figure 5-48). This reflex remained unchanged following the procedure amongst the non-responders, which would be consistent with intact renal sympathetic efferent nerves and inadequate ablation. In contrast, following denervation, the responders demonstrated a trend towards an increase in RBF with handgrip, and although the change in RVR was not significant, the data move towards a fall (rather than an increase) in vascular resistance. These changes would be consistent with disruption of the sympathetic nerves. In contrast to Mahfoud's data (Mahfoud, Cremers et al. 2012), RRI remained unchanged throughout the procedure.

DiBona's group investigated the responses to tail compression and heat stress in rats in the context of RDN (Dibona 2000). In intact animals, these stressors generated an increase in renal vascular resistance, with a fall in RBF. In animals with renal denervation there was no change in RVR or RBF in response to either stressor. Baseline RVR was at a similar level in both intact and denervated animals consistent with our findings in humans. The changes in RBF in this study differ from our data, but the principle that renal denervation can impact the sympathetic control of renal artery tone holds true.

The practical question is whether handgrip stress can be used in the individual patient, at the time of the procedure, to determine adequate renal nerve disruption? Across the full cohort, there was a significant correlation between the percentage change in RVR with handgrip post-RDN and the change in SBP at 1 month (see Figure 5-47); patients responding to RDN, and therefore likely to have received sufficient denervation, tended to have a decrease in RVR in response to sympathetic stress measured immediately after renal nerve ablation. Nominally, if you consider a BP reduction of ≥10 mmHg at one month as a successful denervation, the linear regression of our data would suggest that individuals with at least a 7.5% reduction in RVR in response to handgrip when stressed are likely to have received sufficient denervation. The difficultly in applying this to clinical practice is that there was no clear cut-off point to indicate adequate denervation, indeed there was a reduction in RVR with handgrip in one of the renal arteries from a patient who failed to respond to RDN.

It is pertinent to consider the potential impact of increased RBF on the kidney. Data from large trials and registries show no adverse effect on renal function post-RDN (Bhatt, Kandzari et al. 2014, Bohm, Mahfoud et al. 2015). Further research is required to establish whether any changes in renal haemodynamics persist in the long term, in the

face of possible reinnervation. Previous studies would indicate that the decrease in RVR lasts out to at least six months (Mahfoud, Cremers et al. 2012, Ott, Janka et al. 2013, Doltra, Hartmann et al. 2016), furthermore, Ott et al. reported that renal perfusion was maintained despite increased RVR, supporting a protective role for local autoregulatory mechanisms (Ott, Janka et al. 2013). The fact that RRI was unchanged across our dataset, despite changes in perfusion pressure, also supports autoregulation.

5.5.4.2 Response of the afferent renal nerves to intra-renal arterial adenosine infusion

Reflexes transmitted following stimulation of afferent renal nerves represent an alternative approach for assessing the integrity of renal nerves. In this study, adenosine was used as an afferent renal nerve stimulus. Adenosine is released in the kidney in response to hypoxia and has a variety of actions (Katholi and Woods 1987, Biaggioni 1992). Data from an in vivo rabbit preparation have shown that the adenosine sensitive neurons lie predominantly in the renal pelvis and are activated within 1-3 minutes of renal arterial adenosine injection, with a response lasting 2-6 minutes, and transmitted in part via A1 receptors (although an alternative/parallel mechanism would be via activation of renal mechanoreceptors due to increased hydrostatic pressure as a result of a local action of adenosine inducing renal vasoconstriction) (Ma, Liu et al. 2004).

In previous animal studies, Katholi demonstrated a rise in systemic blood pressure within 1 minute in response to intra-renal artery adenosine in conscious dogs, and this affect was blunted following renal denervation (Katholi, Hageman et al. 1983, Katholi, McCann et al. 1985). We looked to replicate this finding in humans. The first stage of the afferent nerve testing study was an adenosine dose titration which aimed to find the 'target' dose to achieve a 10-20 mmHg rise in SBP. As can be seen from Figure 5-49, the response to the adenosine titration was highly variable. The median target dose was 30 mcg/kg/min, however a third of patients did not increase systemic SBP in response to any dose of adenosine and reached a maximum titration dose of 140 mcg/kg/min; as the dosage reached higher levels, blood pressure fell even further. The effective adenosine dose reached during titration did not correlate with RDN outcome.

There was poor reproducibility in the response to adenosine, with a significant difference in the average change in SBP in response to adenosine at the peak titration dose, versus the test dose administered in ipsilateral renal artery after a 3-minute washout. Tolerance to adenosine due to receptor saturation or down-regulation seems unlikely since the receptors would have been aggressively stimulated during the continuous adenosine titration and an SBP rise was observed in the first renal artery during the adenosine infusion administered after the washout period. The cumulative systemic vasodilatory effect of sustained adenosine infusion during the dose titration may have blunted the reflex increase in SBP in response to intra-renal adenosine during the titration, potentially explaining the poor reproducibility, but ultimately the response to adenosine both within, and between, subjects was highly variable.

Intra-renal adenosine has both systemic effects on blood pressure (Katholi, Hageman et al. 1983), and local autoregulatory effects (Wierema, Houben et al. 2005), making evaluation of our renal haemodynamic data in response to adenosine infusion complex. In vitro studies using a blood-perfused rat juxtamedullary nephron preparation, demonstrated afferent and efferent renal arteriolar A1 receptor mediated

vasoconstriction in response to superfusion of 1, 10 and 100 µmol/l adenosine, which was partly buffered by A2a receptor mediated vasodilatation (Nishiyama, Inscho et al. 2001). A1 receptor agonists induce vasoconstriction and activation of the tubuloglomerular feedback system, through which information about sodium ion concentration is transmitted from the downstream renal tubules, to the upstream renal glomeruli (adenosine is released from the macula densa cells located near the distal tubules in response to increased sodium ion concentration, resulting in constriction of the afferent renal arteriole and a decrease in glomerular filtration rate) (Spielman and Arend 1991). Vasodilatation is mediated via the A2a receptors which also inhibit tubular sodium reabsorption (Smits, de Leeuw et al. 1991, Wierema, Houben et al. 2005). In man, intra-renal arterial adenosine administration has been shown to cause both renal vasoconstriction (e.g. in response to 1ml boluses of 10^{-5}-1 mg/ml adenosine (Marraccini, Fedele et al. 1996)) and vasodilatation (at concentrations in the range of 1-10 mcg/kg/min as used in this study (Smits, de Leeuw et al. 1991, Wierema, Houben et al. 2005)). Studies of intrarenal adenosine infusion in man have predominantly focussed on this vasomotor effect, and data on changes in systemic arterial BP in response to adenosine infusion of 1-10 mcg/kg/min have either not been reported (Wierema, Houben et al. 2005), or shown no effect (n=8 (Smits, de Leeuw et al. 1991)).

The pooled data for both renal arteries showed no change in SBP in response to adenosine pre-RDN, but a fall in SBP post-RDN which was contrary to our hypothesis. There are several potential explanations for this:

i. The dose of adenosine used was too high because the titration was too rapid, and vasodilatory effects are seen from over-spill of adenosine into the systemic circulation. This may have occurred if insufficient time was allowed for the adenosine to transit into the renal pelvis (although the 3-minute infusion used in this study should have been sufficient based on previous research (Ma, Liu et al. 2004)), or because modest changes in systemic blood pressure were difficult to confirm over the blood pressure fluctuates in patients under mild conscious sedation in a stressful catheter laboratory situation.
ii. Prior to RDN, the reflex increase in SBP due to activation of the afferent renal nerves balanced the systemic vasodilation from the effect of adenosine on the systemic vasculature, resulting in no net change in SBP. Post-RDN, the reflex increase in SBP was abolished and therefore the hypotensive effect of systemic vasodilation predominated.
iii. Adenosine is a complex stimulus with a variety of local and reflex responses including opposing vasoactive responses in arterioles; A1 receptor-mediated afferent nerve activation, local vasoconstriction and inhibition of renin release, and A2 receptor-mediated vasodilatation (Biaggioni 1992).
iv. Variable circulating levels of midazolam and fentanyl over the course of the procedure could have acted to lower systemic pressure and suppress renal afferent mediated reflex responses in SBP (see Figure 5-43).

The data were also analysed independently for the first and second renal arteries denervated. The BP response to the adenosine stimulus was evaluated in the first renal artery, before and after denervation, and then the operator moved on to assess the response to adenosine in, and denervation of, the second renal artery. In the first renal artery, prior to RDN, there was a (non-significant) rise in SBP, with a significant reduction

in SBP in response to adenosine after denervation (see Figure 5-52). This would be consistent with the second explanation above; the rise in SBP in response to adenosine is blunted by systemic vasodilatation prior to denervation when the afferent arm of the reflex in intact, but there is a hypotensive response to adenosine post-RDN due to unopposed systemic vasodilation resulting from disruption of the afferent renal nerves. This would support our hypothesis; however, the explanation is contradicted by the significant reduction in SBP in response to adenosine in the second renal artery prior to RDN when the renal afferent mediated hypertensive response to adenosine should remain intact (see Figure 5-52).

The interaction between the two kidneys is complex, the afferent nerves are not disrupted in isolation, with denervation also affecting the efferent nerves, as well the potential for spill-over of adenosine into the contralateral renal artery at higher infusion rates. Afferent renal nerve activation generally has an inhibitory effect on the contralateral kidney via reno-renal reflexes, reducing efferent sympathetic nerve activity in the contralateral kidney to minimise sodium retention and reduce RVR (Kopp 2015). Activation of the reno-renal reflex in the contralateral kidney in response to adenosine may have an antihypertensive effect which nulls the rise in systemic BP caused by adenosine stimulation of the ipsilateral afferent nerves, however, given that the reno-renal reflex is also disrupted during denervation, this does not explain the fall the SBP post-RDN. Furthermore, in hypertension, there is evidence for a shift from inhibitory to excitatory reno-renal reflexes, potentially activated by adenosine, which could contribute to an increase in arterial pressure (Kopp 2015). Unfortunately, in this study, the changes in RBF were not recording in the contralateral renal artery during adenosine infusion and so it is difficult to evaluate this interaction.

Exploring the data by RDN BP outcome group, neither responders nor non-responders had a change in BP in response to adenosine before denervation when the afferent nerves were intact, potentially reflecting the balance between the reflex increase in SBP due to activation of the afferent renal nerves and systemic vasodilation from the effect of adenosine on the peripheral vasculature. However, following denervation, there was no BP response to adenosine amongst responders, and it was the non-responders who showed a decrease in SBP with adenosine post-RDN when we would have predicted there their response to the stimulus would remain unchanged if the nerves were not adequately ablated. The trends towards an increase in RBF and decrease in RVR in response to adenosine post-RDN were also localized to the non-responder group, and difficult to explain as no change would be expected if their renal nerves remained intact. These contradictory findings may represent the small sample size in our cohort, with only a few individuals having complete adenosine testing data for comparison before and after the procedure or raise the possibility that the BP outcomes recorded in the study are not related to renal nerve ablation.

Prior to RDN the bilateral pooled data showed a percentage increase in RVR in response to adenosine (see Table 5-34), potentially due to a direct vasoconstrictor effect mediated via stimulation of the A1 receptors, and/or via reflex mediated activation of the sympathetic efferent nerves. After denervation this pattern was reversed, with a tendency towards a decrease RVR and increase in RBF, potentially due to unmasking of A2 receptor stimulation in the efferent arteriole in the absence of renal afferent reflex mediated sympathoexcitation and concurrent efferent denervation (Spielman and Arend

1991). These findings may also reflect autoregulation within the kidney to maintain perfusion in the face of the systemic hypotensive response to adenosine seen post-RDN.

When looking at the haemodynamic responses to adenosine infusion, the only parameter measured prior to RDN which correlated with the 1-month BP outcome was the change in renal artery diameter in response to this stimulus; those who vasoconstricted in response to adenosine were unlikely to respond to RDN. It may be that these individuals have physiology based on stronger local autoregulation, with renal afferent nerves that were not functional or not producing a pressor response, hence they were not responsible for the hypertension in these patients. A fall in renal artery diameter of >5% pre-RDN, would indicate that the patient would not respond to denervation based on this cohort, however, numbers are small, and measurement of vessel diameter using 2-dimensional fluoroscopic imaging may be prone to error. Furthermore, this technique is invasive and any test to predict a patient's response to RDN should ideally be performed non-invasively to avoid an unnecessary procedure. The use of any parameter to predict an individual's response after denervation may be of academic interest, but would not prevent unnecessary ablation, and the aim of this study was to develop a test to guide the operator as to whether adequate denervation has been achieved at the time of the procedure (or whether further ablation should be administered) rather than to predict an individual's likelihood of response.

5.5.4.3 Limitations

There were multiple acknowledged limitations to this study. First, the small sample size makes it difficult to draw any firm conclusions. More research is required, particularly into the potential for handgrip stress as a tool for assessing procedural efficacy. Second, the end-point for RDN response was based on a 1-month office SBP; the patients did all have ABPM at baseline and follow-up, but in two patients the high cuff inflation pressures could not be tolerated for the full 24hr measurement period, and in one patient, BP was so high that the automated device was unable to record data. Third, a positive aspect of the study was that the investigations and denervation procedure were performed under mild conscious sedation, as is standard practice for clinical RDN. The patients were indeed alert enough to perform isometric handgrip. However, the different levels of sedation through the procedure introduced a confound into the data not seen in the animal work by Tsioufis et al., which took place under general anaesthesia (Tsioufis, Papademetriou et al. 2013). Finally, in the analysis of our data we have made the assumption that those patients who have 'responded' to denervation have had an anti-hypertensive effect secondary to the disruption of their renal nerves. If their BP reduction is due to other factors (e.g. increased medication adherence and a Hawthorne effect), then any relationships inferred from the correlation between our data and the blood pressure outcome would be inaccurate.

5.5.4.4 Future Directions

Handgrip stress as an efferent nerve stimulus show promise as a tool for assessing the efficacy of renal denervation and warrants investigation in a larger study to confirm the reproducibility of our findings and establish a cut-off to be used to guide ablation therapy.

The use of an electrical renal nerve stimulus has generally mandated the use of general anaesthesia (although the new custom designed ConfidenHT RNS system has been used under conscious analgosedation (Tsioufis, Dimitriadis et al. 2018)), and thus the search for alternative modalities and chemical agents to act as afferent renal nerve stimuli continues. Adenosine has diverse effects of the kidney and renal vasculature which makes interpretation of our data a challenge. In rats, infusion of bradykinin into the ipsilateral renal artery induced an immediate increase in MAP, heart rate and both ipsi- and contralateral renal resistance; this effect was abolished by ipsilateral renal denervation (Smits and Brody 1984). Intra-renal bradykinin also stimulates an excitatory reno-renal reflex which can be disrupted by afferent denervation in rats (Barry and Johns 2015). It may therefore be of benefit to trial bradykinin, or a cocktail of renal afferent stimuli, as an alternative afferent stimulus.

A pragmatic approach to inadequate renal denervation is to increase the number of ablation points applied to the renal arteries, and, in the light of recent evidence, to direct therapy to the distal main and branch renal arteries (Sakakura, Ladich et al. 2014, Mahfoud, Tunev et al. 2015, Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018). Review of novel anatomical human data indicates that the renal nerves accessible to intraluminal RF energy lie more distally in the renal artery adventitia (Sakakura, Ladich et al. 2014). The current RDN catheters have a depth of ablation of 2-4 mm, however, whilst the density of renal nerves is highest in the proximal segment of the renal artery, the nerves in the distal segment are closer to the lumen, with 75% of nerves lying within the first 3mm in the distal region compared with only ~50% lying inside this range in the proximal and middle segments; therefore operators following earlier guidance to focus ablation on the proximal-superior aspect of the renal artery, may have been targeting the wrong part of the vessel (Mahfoud, Edelman et al. 2014). Preliminary data from the SPYRAL HTN studies may suggest that more intensive ablation of the main and branch renal arteries achieves a clinically significant reduction in SBP, and support a pragmatic, aggressive ablation approach to RDN (Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018). In this study there was an inverse correlation between then number of ablation points and the SBP reduction following RDN, and interestingly, Fudim et al. argue that with the positive outcomes using electrical RNS, it is a targeted approach to RDN that is required to try to eliminate the heterogeneous BP response to RDN (Fudim, Sobotka et al. 2018).

5.5.5 Conclusions

The clinical effectiveness of RDN in the control of hypertension awaits confirmation from ongoing large-scale clinical trials. However, a simple test to measure afferent and/or efferent renal nerve integrity at the time of the procedure would provide an invaluable tool to establish whether treatment failure is due to inadequate denervation, or other patient or physiological factors. The measurement of changes in renal blood flow and renal vascular resistance in response to dynamic handgrip stress shows potential for this application. An inability to increase RVR with handgrip after denervation may indicate disruption of the renal sympathetic nerves, although the technique may lack sensitivity to guide ablation in the individual patient. Tests to differentiate between afferent and efferent renal nerve function would provide useful

additional information about the mechanisms underlying any successful anti-hypertensive effect of RDN. Unfortunately, adenosine as trialled in this sub-study, is not an adequate afferent stimulus. We were unable to reliably replicate in all patients the reflex increase in systemic blood pressure in response to intra-renal artery adenosine infusion seen in animal models(Katholi, Hageman et al. 1983), and the heterogeneric local and systemic effects of adenosine introduce stimulus confounds that prevented a clear measure of afferent nerve integrity. A pragmatic approach is likely to be the best way forward, with more aggressive ablation in the distal and branch renal arteries.

5.6 Predictors of blood pressure response to renal denervation

5.6.1 Introduction

As demonstrated by the blood pressure outcomes in this study (see Section 5.2.3.2), along with previously published data, the antihypertensive effect of renal denervation can differ significantly between individuals (Esler, Krum et al. 2010, Bhatt, Kandzari et al. 2014, Townsend, Mahfoud et al. 2017). RDN is an invasive procedure, and it would be clinically useful to be able to identify those individuals most likely to benefit from this intervention with a reduction in blood pressure. Physiologically, is a subject likely to respond to a treatment targeting a reduction in afferent and/or efferent renal nerve activity and ultimately a reduction in systemic sympathetic nerve activity (SNA)? If hypertension in a particular patient is not driven by raised SNA, then they may be unlikely to respond to RDN.

Conventionally a BP response to RDN has been arbitrarily defined as a reduction in oSBP of ≥10mmHg (Krum, Schlaich et al. 2009), and studies have looked to identify predictors of response to RDN. The strongest positive predictor for a reduction in office systolic BP (oSBP) in the Symplicity HTN-3 study was a baseline oSBP of ≥180 mmHg (Bhatt, Kandzari et al. 2014, Kandzari, Bhatt et al. 2015), a criterion which has previously been shown to correlate with BP reduction post RDN, as highlighted in the Global Symplicity and Heidelberg registry data (Vogel, Kirchberger et al. 2014, Bohm, Mahfoud et al. 2015). Further to this, Persu et al. looked at the outcome measure used to determine a response to RDN: when office BP was used to define response, responders were more likely to have white coat hypertension, with no effect on ABPM measures, but when ABPM was used to define response to RDN, baseline ABPM did predict a reduction in ABPM following the procedure (Persu, Azizi et al. 2014). Through further interrogation of ABPM data, the same group also demonstrated a reduction in BP variability following RDN (independent of absolute BP level), and that baseline DBP variability correlated with the reduction in mean DBP following RDN (Persu, Gordin et al. 2018).

Arterial stiffness has also been reported as a clinical predictor of response to RDN. Ewen et al. reported that patients with isolated systolic hypertension (ISH) and therefore lower DBP, have a restricted response to RDN (Ewen, Ukena et al. 2015), and these findings were supported by preliminary data from our Bristol CardioNomics cohort combined with data from St Bartholomew's Hospital in London (Burchell, Chan et al. 2016) and by data pooled from the Symplicity HTN-3 cohort and the Global Symplicity registry (Mahfoud, Bakris et al. 2017), thus indicating that ISH with potentially stiffer arteries, may be less likely to respond to RDN. Fengler et al. further expanded on this concept by quantifying pulse wave velocity (PWV) as a measure of vascular stiffness in patients with ISH undergoing RDN, and reported that ISH patients with the lowest tertile of PWV had a comparable response to RDN as those with combined systolic and diastolic hypertension (Fengler, Rommel et al. 2017). A lower invasive aortic PWV, lower non-invasively measured central pulse pressure and a lower ambulatory aortic stiffness index were all predictors of a BP reduction following RDN (Ott, Schmid et al. 2015, Okon, Rohnert et al. 2016, Sata, Hering et al. 2018). Conversely, individuals with higher baseline aortic distensibility have been reported to be more likely to respond to RDN (Fengler, Rommel et al. 2018). Baseline LV wall thickness, another marker of target organ damage, has been reported to predict the reduction in myocardial mass post-RDN

(Ripp, Mordovin et al. 2015). Thus, whilst the initial report from Symplicity HTN-3 that younger age may predict response to RDN, which has not been substantiated in wider studies, it may be that neuro-haemodynamic factors play a greater role in the regulation of blood pressure in patients with lower vascular resistance, making them more susceptible to RDN and autonomic modulation, as opposed to individuals with stiff arteries and more significant target organ damage in whom biomechanical factors may have a greater role to role in driving hypertension (Barber-Chamoux and Esler 2017).

Investigators have also looked at vascular and inflammatory markers to predict response to RDN, with preliminary data supporting the predictive value of the profibrotic marker Galectin-3 (Schwerg, Eilers et al. 2016), angiogenic factors including soluble fms-like tyrosine kinase-1 (sFLT-1), intercellular cell adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) (Dorr, Liebetrau et al. 2014), and the cardiovascular prognostic marker MR-proadrenomedullin (Neumann, Schwerg et al. 2016). Steinmetz et al. have reported that assessment of endothelial function through flow-/nitro-glycerine-mediated dilation measurements demonstrates that patients with more endothelial dysfunction and a higher vasomotor tone are also more likely to respond to RDN (Steinmetz, Nelles et al. 2018). Low vitamin D levels have also been associated with a poor response to RDN (Poss, Mahfoud et al. 2014). All of the factors reflect small-scale data and require further substantiation.

Measurement of renal SNA would be an ideal variable to test the mechanism of action of RDN, and potentially to predict the likelihood of response to an intervention targeting this pathway. It is not feasible to assess renal SNA on a clinical basis since organ specific measurement of noradrenaline spillover is not widely available. Muscle SNA (MSNA) gives a relatively more accessible measure of SNA, but it must be remembered that MSNA is only a surrogate for efferent RSNA or for the impact of changes in afferent renal nerve activity on systemic SNA. MSNA has not yet been formally assessed as a predictor of response to RDN (Barber-Chamoux and Esler 2017), however, the studies investigating the effect of RDN on MSNA that have reported the association between baseline MSNA and BP outcomes have not identified a significant relationship between these variables (Hering, Marusic et al. 2014, Grassi, Seravalle et al. 2015). Initial data on an association between baseline baroreflex sensitivity and the BP response to RDN have been conflicting, with Zuern et al. reporting that impaired baseline cardiac baroreflex sensitivity identified RDN-responders (Zuern, Eick et al. 2013), whilst Grassi et al. showed no association between baseline sympathetic BRS and the change in ABPM post-RDN (Grassi, Seravalle et al. 2015)

In this pilot study, we aimed to build on possible clinical cardiovascular predictors of response and to use our comprehensive autonomic profiling protocol to identify potential predictors of a BP response to RDN.

5.6.2 Methods

Baseline measures and office BP outcomes measures were quantified as described in preceding sections.

5.6.2.1 Univariate analyses

Datasets were assessed for normality using a Shapiro-Wilk normality test. Based on this, baseline data were correlated against 6-month office systolic blood pressure (oSBP) outcome data using either a Pearson's correlation coefficient for normally distributed data, or a Spearman's correlation coefficient for all other continuous data. Where there was a significant correlation ($p<0.05$), a linear regression of the data was performed to further describe the relationship (GraphPad Prism, GraphPad Software Inc. La Jolla, CA, USA).

The differences in baseline variables between RDN BP responders and non-responders were assessed for significance using a Student's T-test. A BP response to RDN was defined as a reduction in oSBP of ≥10 mmHg at 6 months post-RDN. A Fisher's exact test was used to quantify any difference in categorical baseline variables between response groups (GraphPad Prism, GraphPad Software Inc. La Jolla, CA, USA). A reduction in office SBP was used at the primary outcome measure for this study data for this variable was available in all 18 study participants at both baseline and 6 months post-RDN.

Baseline variables of interest were identified from the above analyses and further assessed using a binary logistic regression to quantify the likelihood of each variable affecting the response to RDN (SPSS Statistics, Version 24 IBM Corp., Armonk, NY, USA). The dependent variable was renal denervation BP response or non-response at 6 months post-procedure. Data were analysed using a binary logistic regression model using an enter method. These data are reported in Appendix 4.

5.6.2.2 Multivariate analyses

Multivariate analyses were considered, but due to the small numbers of patients in this pilot study the analyses were felt to be underpowered and not statistically robust.

5.6.3 Results

5.6.3.1 Univariate analyses

5.6.3.1.1 Baseline clinical outcome measures

There was a significant correlation between the change in oSBP at 6 months post-RDN and both baseline oSBP (see Figure 5-54) and office mean arterial pressure (oMAP), but not versus baseline office pulse pressure (oPP) (see Table 5-35). This was also seen as a significant difference between baseline oSBP and oMAP between RDN responders and non-responders (see Table 5-35). There were no correlations between baseline ABPM parameters and the change in oSBP at six months post-RDN, and no difference in baseline ABPM parameters between responders and non-responders (see Table 5-36).

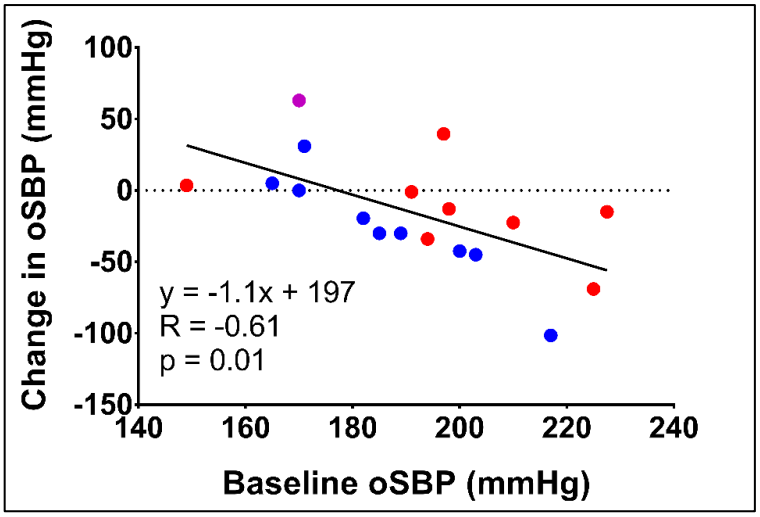


Figure 5-54. Correlation between baseline office systolic blood pressure (oSBP) and the change in oSBP six-months after renal denervation.
Patients with a higher baseline oSBP had a greater reduction in oSBP following RDN. Significant linear regression and Pearson's correlation coefficient shown. Data for premenopausal females are shown in red, postmenopausal females are shown in purple and males are shown in blue.

Baseline parameter	Correlation		RDN response group		
	R	P	Responders	Non-responders	P
Age (years)	0.07	0.78	53 ± 3	57 ± 5	0.47
Gender (%male)	-	-	55 (6/11)	43 (3/7)	1.00
BMI (kg/m^2)	0.06	0.81	28.4 ± 0.6	29.1 ± 1.5	0.64
oSBP (mmHg)	-0.61	0.01	203 ± 5	173 ± 6	0.002
oDBP (mmHg)	-0.35	0.16	113 ± 5	94 ± 10	0.11
oMAP (mmHg)	-0.48	0.04	143 ± 4	120 ± 8	0.03
oPP (mmHg)	-0.24	0.33	90 ± 7	79 ± 6	0.28
HR (beats/min)	-0.09	0.71	69 ± 2	62 ± 4	0.15
TPR	-0.09	0.71	20 ± 1	20 ± 2	0.92

Table 5-35. Correlations between the office systolic blood pressure (oSBP) response to renal denervation (RDN) 6 months after renal denervation (RDN) and baseline clinical parameters.
The correlation data is for the whole cohort of 18 patients with the data given for the Pearson/Spearman coefficient R value and level of significance (p). Data are also given by RDN BP response group, with data shown for responders (n=11) and non-responders (n=7; mean ± SEM) and the p value from a Student's t-test between the baseline data shown for the two response groups. Fisher's exact test was used to quantify any difference between categorical data. BMI; body mass index, oDBP; office diastolic blood pressure, oMAP; office mean arterial pressure, oPP; office pulse pressure, HR; heart rate, TPR; total peripheral resistance.

	Correlation	RDN response group

Baseline parameter	n	R	P	N	Responders	N	Non-responders	P
Day SBP (mmHg)	13	-0.25	0.41	6	173 ± 7	7	169 ± 4	0.66
Day DBP (mmHg)	13	-0.36	0.23	6	105 ± 4	7	94 ± 6	0.13
Day MAP (mmHg)	10	0.12	0.74	5	122 ± 4	5	120 ± 4	0.80
Day PP (mmHg)	10	0.17	0.63	5	67 ± 8	5	79 ± 6	0.25
Day HR (beats/min)	10	0.12	0.75	5	76 ± 6	5	72 ± 6	0.66
Night SBP (mmHg)	12	-0.16	0.61	6	154 ± 8	6	158 ± 7	0.68
Night DBP (mmHg)	12	-0.21	0.51	6	88 ± 4	6	84 ± 6	0.64
Night MAP (mmHg)	10	0.01	0.98	5	111 ± 8	5	109 ± 3	0.82
Night PP (mmHg)	10	-0.04	0.91	5	66 ± 7	5	77 ± 12	0.45
Night HR (beats/min)	10	0.21	0.57	5	65 ± 1	5	65 ± 5	0.92
24hr SBP (mmHg)	10	0.04	0.92	5	164 ± 8	5	168 ± 3	0.68
24hr DBP (mmHg)	10	-0.35	0.32	5	97 ± 2	5	90 ± 7	0.35
24hr MAP (mmHg)	10	0.16	0.65	5	119 ± 4	5	118 ± 3	0.90
24hr PP (mmHg)	10	0.12	0.75	5	67 ± 7	5	78 ± 7	0.29
24hr HR (beats/min)	10	0.15	0.67	5	73 ± 4	5	70 ± 6	0.75

Table 5-36. No correlations between the change in office systolic blood pressure 6 months after renal denervation (RDN) and baseline ambulatory blood pressure monitoring (ABPM) parameters.

The n number for each analysis is specified with the data given for the Pearson/Spearman coefficient R value and level of significance (p). Data are also given by RDN BP response group, with data shown for responders and non-responders (mean ± SEM) and the p value from a Student's t-test between the baseline data shown for the two response groups. SBP; systolic blood pressure, DBP; diastolic blood pressure, MAP; mean arterial pressure, PP; pulse pressure, HR; heart rate.

There were no correlations between either the number of baseline antihypertensive medications, drug classes or medication whole dose equivalents (WDE) and the change

in oSBP at 6 months post-RDN. These parameters, and the proportions of patients taking each class of antihypertensive medication at baseline did not differ between RDN responders and non-responders (see Table 5-37).

Baseline parameter	Correlation		RDN response group		
	R	P	Responders	Non-responders	P
No. drugs (n)	-0.12	0.64	5.5 ± 0.6	4.7 ± 0.6	0.39
No. classes (n)	-0.03	0.90	4.8 ± 0.6	4.7 ± 0.6	0.90
WDE (n)	-0.21	0.41	4.6 ± 0.8	3.1 ± 0.7	0.14
ACEi/ARB/RI (%)	-	-	100 (11/11)	100 (7/7)	1.00
CCB (%)	-	-	64 (7/11)	71 (5/7)	1.00
Diuretic (%)	-	-	82 (9/11)	57 (4/7)	0.33
MRA (%)	-	-	55 (6/11)	57 (4/7)	1.00
β-Blocker (%)	-	-	64 (7/11)	57 (4/7)	1.00
α-Blocker (%)	-	-	64 (7/11)	57 (4/7)	1.00
Centrally Acting (%)	-	-	36 (4/11)	43 (3/7)	1.00
Vasodilator (%)	-	-	18 (2/11)	29 (2/7)	1.00

Table 5-37. No difference in baseline medication profile between renal denervation (RDN) responders and non-responders.
The correlation data is for the whole cohort of 18 patients with the data given for the Pearson coefficient R value and level of significance (p). Data are also given by RDN BP response group, with data shown for responders (n=11) and non-responders (n=7; mean ± SEM) and the p value from a Student's t-test between the baseline data shown for the two response groups. Fisher's exact test was used to quantify any difference between categorical data. WDE; whole dose equivalent, ACEi; angiotensin converting enzyme inhibitor, ARB; angiotensin receptor blocker, RI; renin inhibitor, CCB; calcium channel blocker, MRA; mineralocorticoid receptor antagonist.

5.6.3.1.2 Baseline target organ damage

There were no significant differences between baseline measures of target organ damage, including markers of renal injury, aortic distensibility and cardiac volumetrics, function and fibrosis, between RDN BP responders and non-responders (see Table 5-38). However, when looking at correlations between the changes in oSBP at 6 months post-RDN and these baseline parameters, there were correlations between baseline ejection fraction (EF), baseline end systolic volume (ESV, borderline correlation for indexed ESV)), and borderline correlations for baseline peak circumferential strain and peak systolic circumferential strain rate, versus the change in oSBP at 6 months (see Table 5-38). To summarise, a lower baseline EF but a higher baseline ESV, and a lower peak circumferential strain or peak systolic circumferential strain rate, were associated with a greater reduction in oSBP following RDN (see Figure 5-55).

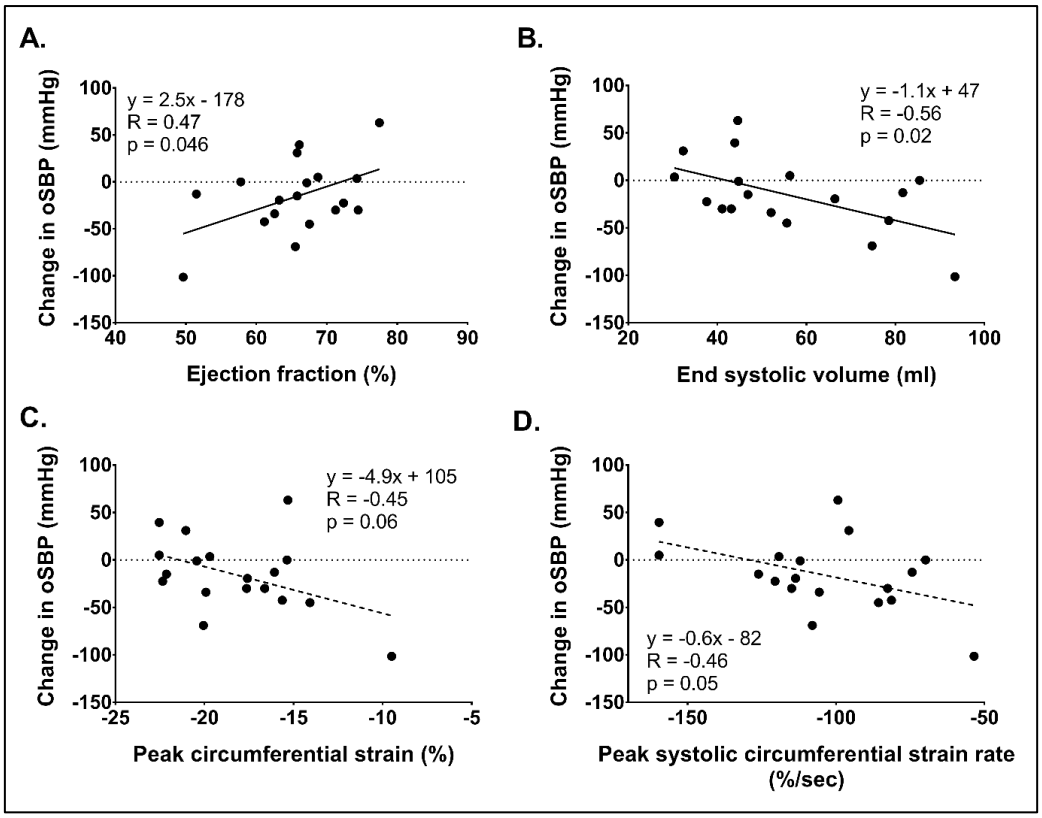


Figure 5-55. Correlations between baseline cardiac volumetric and strain parameters and the change in office systolic blood pressure (oSBP) six-months after renal denervation.

Patients with a higher baseline ejection fraction (A.) and lower end systolic volume (B.) were less likely to response to renal denervation with a reduction in oSBP at 6 months. There were also trends towards individuals with lower (less negative) peak circumferential strain (C.) and peak systolic circumferential strain rate (D.) having a greater reduction in oSBP following RDN.

Baseline parameter	Correlation			RDN response group				
	n	R	P	N	Responders	N	Non-responders	P
eGFR (ml/min/1.73m^2)	18	0.07	0.79	11	74 ± 4	7	72 ± 3	0.70
Albumin:creatinine ratio	15	-0.18	0.53	8	5.7 ± 2.3	7	10.6 ± 7.1	0.53
Aortic distensibility (mm^2/mmHg x10^3)	15	-0.12	0.67	9	1.8 ± 0.4	6	1.2 ± 0.4	0.30
EF (%)	18	0.47	0.046	11	65 ± 2	7	6 ± 3	0.52

LV mass (g)	18	-0.12	0.62	11	176 ± 11	7	180 ± 31	0.90
Indexed LV mass (g/ml)	18	-0.10	0.69	11	87 ± 5	7	94 ± 13	0.62
EDV (ml)	18	-0.36	0.14	11	166 ± 7	7	154 ± 17	0.55
Indexed EDV (ml/m^2)	18	-0.32	0.19	11	83 ± 3	7	80 ± 7	0.78
ESV (ml)	18	-0.56	0.02	11	59 ± 5	7	51 ± 8	0.46
Indexed ESV (ml/m^2)	18	-0.42	0.08	11	29 ± 3	7	27 ± 4	0.62
SV (ml)	18	-0.06	0.82	11	107 ± 5	7	103 ± 11	0.76
Indexed SV (ml/m^2)	18	0.01	0.96	11	53 ± 2	7	53 ± 4	0.96
Peak radial strain (%)	18	0.40	0.10	11	31 ± 3	7	33 ± 3	0.67
Peak circumferential strain (%)	18	-0.45	0.06	11	-18 ± 1	7	-19 ± 1	0.75
Peak longitudinal strain (%)	18	-0.26	0.29	11	-18 ± 1	7	-18 ± 1	0.72
Peak systolic radial strain rate (%/sec)	18	0.38	0.12	11	192 ± 23	7	202 ± 23	0.75
Peak systolic circumferential strain rate (%/sec)	18	-0.46	0.05	11	-104 ± 9	7	-105 ± 11	0.93
Peak systolic longitudinal strain rate (%/sec)	18	-0.38	0.12	11	-95 ± 5	7	-102 ± 12	0.66
Peak diastolic radial strain rate (%/sec)	18	-0.31	0.21	11	-200 ± 26	7	-202 ± 34	0.96
Peak diastolic circumferential strain rate (%/sec)	18	0.27	0.28	11	111 ± 13	7	109 ± 20	0.94
Peak diastolic longitudinal strain rate (%/sec)	18	0.36	0.15	11	121 ± 15	7	105 ± 19	0.51
Extracellular volume fraction	7	-0.14	0.77	3	0.27 ± 0.01	4	0.26 ± 0.01	0.59
Interstitial volume (ml)	7	0.15	0.75	3	44 ± 8	4	54 ± 16	0.61
Index interstitial volume (ml/m^2)	7	0.08	0.87	3	22 ± 2	4	27 ± 7	0.49
Myocardial cell volume (ml)	7	0.20	0.66	3	116 ± 13	4	145 ± 37	0.51
Index myocardial cell volume (ml/m^2)	7	0.13	0.78	3	58 ± 3	4	75 ± 15	0.35

Table 5-38. No difference in baseline marker of target organ damage between renal denervation (RDN) responders and non-responders.
Baseline data for renal impairment, aorta distensibility/vascular stiffness and hypertensive heart disease as assessed by cardiac MRI did not correlate with the change in oSBP 6-month post-RDN. The n number for each analysis is specified with the data given for the Pearson/Spearman coefficient R value and level of significance (p). Data are also given by RDN BP response group, with data shown for responders and non-responders (mean ± SEM) and the p value from a Student's t-test between the baseline data shown for the two response groups. eGFR; estimated glomerular filtration rate, EF; ejection fraction, LV; left ventricle, EDV; end diastolic volume, ESV; end systolic volume, SV; stroke volume.

5.6.3.1.3 Baseline sympathetic nerve activity

There were no correlations between either baseline muscle sympathetic nerve activity (MSNA) or markers of baseline cardiac sympathetic nerve activity (as assessed by heart rate variability) and the change in oSBP 6-months post-RDN. There were also no differences in these parameters between RDN responders and non-responders at baseline (see Table 5-39).

Baseline parameters	Correlations			RDN response group				
	n	R	P	n	Responders	n	Non-responders	P
MSNA incidence (bursts/100 HB)	14	0.05	0.87	8	55 ± 6	6	67 ± 10	0.34
MSNA frequency (bursts/min)	14	0.01	0.98	8	37 ± 5	6	39 ± 4	0.72
Total MSNA area/100 HB (%/s)	14	0.17	0.57	8	2790 ± 350	6	3886 ± 631	0.17
Total MSNA area/min (%/s)	14	0.17	0.56	8	1880 ± 243	6	2273 ± 259	0.29
Transduction (mmHg/%.s)	13	-0.14	0.64	8	0.01 ± 0.04	5	0.06 ± 0.08	0.60
SDNN (ms)	17	-0.11	0.68	10	42 ± 7	7	46 ± 6	0.69
RMSSD (ms)	17	0.26	0.32	10	31 ± 6	7	48 ± 12	0.23
NN50 (n=)	17	0.13	0.63	10	13 ± 5	7	26 ± 12	0.33

pNN50 (%)	17	0.15	0.55	10	4 ± 1	7	8 ± 3	0.27
Total power (ms2)	17	0.12	0.64	10	1987 ± 599	7	2119 ± 563	0.87
VLF (ms2)	17	0.13	0.63	10	988 ± 355	7	605 ± 90	0.32
LF (ms2)	17	0.25	0.34	10	325 ± 85	7	602 ± 221	0.27
nLF (n.u.)	17	0.04	0.88	10	47 ± 9	7	42 ± 10	0.69
HF (ms2)	17	0.17	0.51	10	377 ± 138	7	627 ± 281	0.44
nHF (n.u.)	17	0.17	0.52	10	34 ± 5	7	47 ± 9	0.25
LF/HF	17	-0.01	0.98	10	1.9 ± 0.6	7	2.1 ± 1.3	0.92

Table 5-39. No differences between baseline muscle sympathetic nerve activity (MSNA) and heart rate variability (HRV) parameters between renal denervation (RDN) responders and non-responders.

Baseline data for MSNA, sympathovascular transduction and HRV did not correlate with the change in oSBP 6-month post-RDN. The n number for each analysis is specified with the data given for the Pearson/Spearman coefficient R value and level of significance (p). Data are also given by RDN BP response group, with data shown for responders and non-responders (mean ± SEM) and the p value from a Student's t-test between the baseline data shown for the two response groups. SDNN; standard deviation of differences between successive NN (normal to normal) intervals, RMSSD; square root of the mean squared differences of successive NN intervals, NN50; number of interval differences of successive NN intervals measuring >50 ms, pNN50; NN50 count as a percentage of the total number of all NN intervals, VLF; very low frequency, LF; low frequency, nLF; normalised low frequency, HF; high frequency, nHF; normalised high frequency.

5.6.3.1.4 Baseline baroreflex sensitivity

There was a significant correlation between baseline spontaneous sympathetic baroreflex sensitivity (BRS) as assessed by the area method (ssBRSA) and the change in oSBP 6 months post-RDN, with responders trending towards having greater baseline spontaneous sympathetic baroreflex gain by this technique than non-responders (see Figure 5-56 and Table 5-40). There was a trend towards a correlation between baseline spontaneous sympathetic baroreflex sensitivity to falling diastolic BP (assessed by the threshold method) and the change in oSBP post-RDN, following a similar pattern (see Table 5-40). There were also trends towards a difference in baseline sympathetic BRS for both rising and falling DBP, assessed in response to a Modified Oxford protocol, between RDN responders and non-responders, however patient numbers are too small to place significant emphasis on these latter findings (see Table 5-40). There were no associations between baseline cardiac BRS parameters and the change in oSBP 6-months post-RDN, and no differences between the baseline measures of these parameters between RDN responders and non-responders.

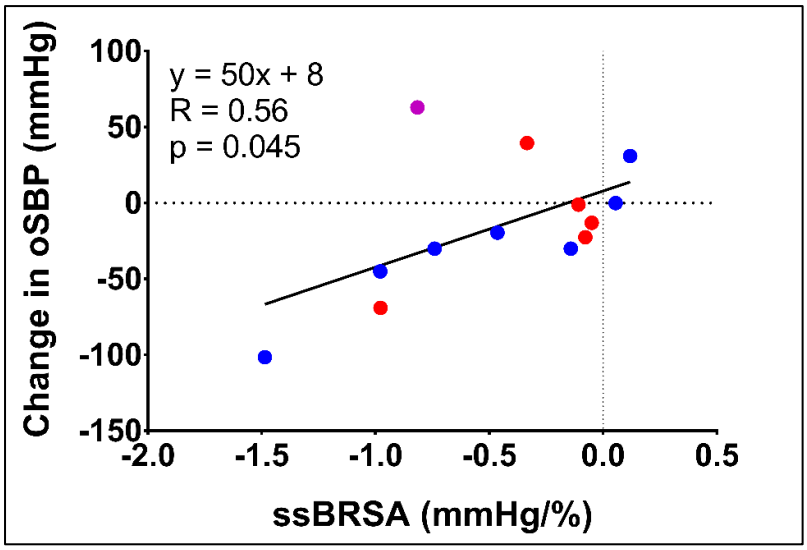


Figure 5-56. Greater baseline spontaneous sympathetic baroreflex sensitivity is associated with a greater blood pressure reduction six-months following renal denervation.

Data is for linear regression and Pearson's correlation coefficient. ssBRSA; spontaneous sympathetic baroreflex sensitivity as assessed by the area method. Data for premenopausal females are shown in red, postmenopausal females are shown in purple and males are shown in blue.

Baseline parameters	Correlations			RDN response group				
	N	R	P	n	Responders	N	Non-responders	P
ssBRST overall (%/mmHg)	13	0.30	0.33	8	-1.52 ± 0.38	5	-1.16 ± 0.5	0.58
ssBRST rising (%/mmHg)	13	0.03	0.91	8	-1.23 ± 0.44	5	-1.41 ± 0.9	0.86
ssBRST falling (%/mmHg)	13	0.52	0.07	8	-1.29 ± 0.33	5	-0.72 ± 0.2	0.16
ssBRSA overall (AU•s/mmHg)	13	0.56	0.045	8	-0.61 ± 0.18	5	-0.22 ± 0.2	0.14
ssBRSA rising (AU•s/mmHg)	13	0.10	0.74	8	-0.68 ± 0.27	5	-0.66 ± 0.4	0.96
ssBRSA falling (AU•s/mmHg)	13	0.46	0.11	8	-0.73 ± 0.20	5	-0.32 ± 0.2	0.22
scBRS overall (ms/mmHg)	13	0.04	0.90	9	7.0 ± 1.6	4	11.3 ± 2.7	0.22
scBRS rising (ms/mmHg)	13	-0.08	0.80	9	6.2 ± 1.4	4	8.7 ± 4.7	0.64
scBRS falling (ms/mmHg)	11	0.29	0.38	8	7.8 ± 2.2	3	17.6 ± 5.9	0.23
BEI overall	12	-0.16	0.62	9	0.21 ± 0.03	3	0.23 ± 0.05	0.73
BEI rising	16	-0.25	0.34	10	0.22 ± 0.04	6	0.17 ± 0.04	0.37
BEI falling	16	-0.23	0.39	10	0.19 ± 0.03	6	0.19 ± 0.07	1.00
psBRSA overall (AU•s/mmHg)	5	0.73	0.16	3	-0.95 ± 0.16	2	-0.54 ± 0.04	0.11

psBRSA rising (AU•s/mmHg)	5	0.74	0.16	3	-0.99 ± 0.15	2	-0.54 ± 0.04	0.09
psBRSA falling (AU•s/mmHg)	5	0.78	0.12	3	-0.66 ± 0.12	2	-0.31 ± 0.03	0.08
pcBRS overall (ms/mmHg)	10	-0.03	0.95	5	3.6 ± 1.3	5	2.4 ± 0.4	0.40

Table 5-40. Correlations between measures of baseline sympathetic and cardiac baroreflex sensitivity versus the change in oSBP 6months post-RDN.
The n number for each analysis is specified with the data given for the Pearson/Spearman coefficient R value and level of significance (p). Data are also given by RDN BP response group, with data shown for responders and non-responders (mean ± SEM) and the p value from a Student's t-test between the baseline data shown for the two response groups. ssBRST; spontaneous sympathetic baroreflex sensitivity-threshold method, ssBRSA; spontaneous sympathetic baroreflex sensitivity-area method, scBRS; spontaneous cardiac baroreflex sensitivity, BEI; baroreflex effectiveness index, psBRSA; pharmacological sympathetic baroreflex sensitivity, pcBRS; pharmacological cardiac baroreflex sensitivity.

5.6.3.1.5 Baseline chemoreflex sensitivity and brain blood flow

When considering parameters which may influence and measure cerebral blood flow (CBF), there were no correlations between baseline measures of hypoxic ventilatory response (HVR; a measure of chemoreflex sensitivity) and the change in oSBP at 6 months post-RDN, and whilst there was a significant difference between baseline HVR as assessed by the intermittent hypoxia method between RDN responders and non-responders, this analysis only included n=3 non-responders and may therefore not be representative of findings in a wider population (see Table 5-41).

There were significant correlations between both the percentage of CBF in the right vertebral artery and the percentage of total cardiac output (CO) flow in the left vertebral artery at baseline versus the change in oSBP at 6-months post-RDN (see Table 5-42). These findings were not mirrored by any significant differences in these, or any of the other baseline CBF parameters, between RDN responders and non-responders, and these findings in different arteries are difficult to rationalise into a mechanism related to RDN. There were also no significant differences between the proportion of responders and non-responders who had either vertebral artery hypoplasia or an incomplete circle of Willis (4/11 responders and 4/7 non-responders, p=0.63).

Baseline parameters	**Correlation**			**RDN response group**				
	N	R	P	n	Responders	N	Non-responders	P
Pooled HVR data (L/min/%)	16	0.17	0.54	9	-0.26 ± 0.08	7	-0.13 ± 0.05	0.20

HVR – intermittent hypoxia method (L/min/%)	10	0.32	0.37	7	-0.34 ± 0.08	3	-0.08 ± 0.03	0.02
HVR – stepped hypoxia method (L/min/%)	6	-0.65	0.17	2	0.02 ± 0.15	4	-0.16 ± 0.08	0.42

Table 5-41. Correlations between baseline measures of chemoreflex sensitivity as assessed by hypoxic ventilatory response (HVR) and the BP response to renal denervation (HVR) at 6 months post-procedure.
The n number for each analysis is specified with the data given for the Pearson/Spearman coefficient R value and level of significance (p). Data are also given by RDN BP response group, with data shown for responders and non-responders (mean ± SEM) and the p value from a Student's t-test between the baseline data shown for the two response groups.

Baseline parameters	**Correlations**			**RDN response group**				
	N	R	P	n	Responders	N	Non-responders	P
RCA (ml/min)	16	-0.37	0.16	10	456 ± 42	6	395 ± 34	0.28
LCA (ml/min)	16	-0.23	0.39	10	409 ± 26	6	396 ± 28	0.75
RVA (ml/min)	16	0.23	0.39	10	121 ± 15	6	128 ± 6	0.67
LVA (ml/min)	16	-0.18	0.50	10	173 ± 19	6	143 ± 19	0.28
Total CBF (ml/min)	16	-0.28	0.29	10	1159 ± 78	6	1062 ± 72	0.38
% RCA flow of CBF	16	-0.39	0.13	10	39 ± 2	6	37 ± 2	0.35
% LCA flow of CBF	16	0.06	0.83	10	36 ± 2	6	37 ± 1	0.36
% RVA flow of CBF	16	0.59	0.02	10	10 ± 1	6	12 ± 1	0.15
% LVA flow of CBF	16	0.44	0.09	10	48 ± 23	6	83 ± 33	0.41
% Total CBF of CO	16	-0.20	0.44	10	16.1 ± 1.6	6	18.4 ± 2.5	0.46
% RCA of CO	16	0.38	0.15	10	6.3 ± 0.7	6	6.8 ± 0.9	0.66
% LCA of CO	16	0.19	0.49	10	5.7 ± 0.6	6	6.8 ± 0.9	0.34
% RVA of CO	16	0.38	0.15	10	1.7 ± 0.2	6	2.3 ± 0.4	0.25
% LVA of CO	16	0.59	0.02	10	2.4 ± 0.3	6	2.6 ± 0.6	0.79

Table 5-42. No difference in baseline cerebral blood flow (CBF) parameters between responders and non-responders to renal denervation (RDN).
The n number for each analysis is specified with the data given for the Pearson/Spearman coefficient R value and level of significance (p). Data are also given by RDN BP response group, with data shown for responders and non-responders (mean ± SEM) and the p value from a Student's t-test between the baseline data shown for the two response groups. RCA; right carotid artery, LCA; left carotid artery, RVA; right vertebral artery, LVA; left vertebral artery, CO; cardiac output.

5.6.3.1.6 Baseline markers of inflammation

There were no significant correlations between baseline markers of inflammation and the change in oSBP at 6 months following RDN. There were also no significant differences between the baseline levels of markers of inflammation between RDN responders and non-responders (see Table 5-43).

Baseline parameters	Correlations			RDN response group				
	n	R	P	N	Responders	n	Non-responders	P
CRP (mg/mL)	9	-0.24	0.53	6	3.3 ± 0.8	3	2.7 ± 1.2	0.67
IL-6 (pg/mL)	9	-0.31	0.42	5	3.4 ± 1.4	4	3.6 ± 1.3	0.94
IL-8 (pg/mL)	9	-0.20	0.61	5	7.8 ± 3.6	4	3.5 ± 0.6	0.30
IL-10 (pg/mL)	9	-0.47	0.20	5	10.2 ± 2.4	4	7.6 ± 2.0	0.42
IL-17 (pg/mL)	9	-0.18	0.64	5	9.2 ± 2.9	4	12.5 ± 3.8	0.52
MPO (pg/mL)	9	0.21	0.59	5	38.2 ± 8.8	4	47.8 ± 15.7	0.62
TNFα (pg/mL)	9	-0.47	0.20	5	10.2 ± 2.4	4	7.6 ± 2.0	0.42

Table 5-43. No correlation between baseline inflammatory markers and the change in office SBP at 6 months post-RDN.
The n number for each analysis is specified with the data given for the Pearson/Spearman coefficient R value and level of significance (p). Data are also given by RDN BP response group, with data shown for responders and non-responders (mean ± SEM) and the p value from a Student's t-test between the baseline data shown for the two response groups. CRP: C reactive protein, IL; interleukin, MPO; myeloperoxidase, TNFα; tumour necrosis factor alpha.

5.6.3.1.7 Binomial regression analyses

Binomial univariate regression analyses were performed for the prediction of response to RDN by baseline age, gender, oSBP, oPP, eGFR, indexed LV mass, aortic distensibility, spontaneous sBRSA, spontaneous cBRS and sympathovascular transduction. These results are summarised in Appendix 4.

5.6.4 Discussion

This study aimed to identify physiological parameters that would predict a blood pressure reduction following RDN, and which could then be used to screen individuals for suitability for this invasive and expensive procedure. The univariate analyses reported above described significant correlations between baseline oSBP, oMAP and spontaneous sympathetic BRS (area method) and the change in oSBP 6 months post-RDN. A greater reduction in BP was associated with a higher baseline oSBP, oMAP and

spontaneous sympathetic BRS (see Figure 5-54 and Figure 5-56). Baseline MSNA did not correlate with change in office SBP following RDN on univariate analysis.

Higher office SBP has previously been reported as a predictor of response to RDN (Bhatt, Kandzari et al. 2014, Vogel, Kirchberger et al. 2014, Bohm, Mahfoud et al. 2015, Kandzari, Bhatt et al. 2015). It has been hypothesised that higher SBP may indicate a greater severity or longer duration of hypertension and thus increased target organ damage and/or vascular stiffness (Barber-Chamoux and Esler 2017). This theory is refuted by the fact there were no correlations between baseline oSBP and either baseline eGFR, baseline aortic distensibility or any of the baseline cardiac volumetric and functional parameters in this cohort. Also contrary to our hypothesis (and data from other groups reported above (Ewen, Ukena et al. 2015, Ott, Schmid et al. 2015, Burchell, Chan et al. 2016, Fengler, Rommel et al. 2017, Mahfoud, Bakris et al. 2017)), was the lack of relationship between either baseline office pulse pressure (increased with increasing vascular stiffness) or baseline aortic distensibility and oSBP outcomes. Symplicity HTN-3 investigators reported that younger participants were more likely to respond to RDN (Bhatt, Kandzari et al. 2014, Kandzari, Bhatt et al. 2015), and it may be that vascular adaptions to hypertension are still reversible in these younger individuals with a greater neuro-haemodynamic component driving up BP (Barber-Chamoux and Esler 2017), however, there was no relationship between age and RDN response in our cohort. These results may reflect our smaller study cohort, but importantly, would not suggest that age, pulse pressure or aortic distensibility could be used as measures to select patients for treatment with RDN on an individual basis.

Our data did not demonstrate an independent correlation between baseline MSNA and the BP response to RDN. MSNA has not previously been formally investigated as a tool to identify patients who are likely to respond to RDN, and whilst in some individuals changes in MSNA did appear to qualitatively track changes in oSBP following RDN (see Figure 5-17), given the significant variability between individuals, our data would not support the use of MSNA to screen for likely responders to RDN. Baseline data and outcome data for the wider cohort are consistent with existing published data showing no direct correlation between changes in BP and MSNA after RDN (Grassi, Seravalle et al. 2014, Hering, Marusic et al. 2014, Vink, Verloop et al. 2014).

Interestingly, subjects with greater baroreflex gain were more likely to respond to RDN (Figure 5-56). Greater baseline spontaneous sympathetic baroreflex sensitivity was associated with higher baseline oSBP and left ventricular mass (LVM) in this cohort, however there were no correlations between changes in oSBP or LVM and changes in BRS following RDN (in fact BRS did not change following RDN, see Section 5.4.1.3). It is possible that these individuals were better able to respond to a change in cardiac afterload driven by mechanisms other than a reduction in MSNA (e.g. local modulation of sodium and water handling in the kidney or renal neurohormonal effects) or were better able to respond to small changes in central sympathetic modulation of BRS not detected in this small pilot study. Further research is required to confirm the reproducibility of this finding in a larger patient population, particularly given the conflicting data from other groups (Zuern, Eick et al. 2013, Grassi, Seravalle et al. 2015).

Patients with a higher baseline ejection fraction and lower end systolic volume were less likely to response to renal denervation with a reduction in oSBP at 6 months, and there were also trends towards individuals with lower (less negative) peak circumferential

strain and peak systolic circumferential strain rate to have a greater reduction in oSBP following RDN. There was no correlation between baseline left ventricular mass and the change in oSBP post-RDN despite reports from Ripp et al. that baseline LV wall thickness predicted the reduction in myocardial mass post-RDN (Ripp, Mordovin et al. 2015). If more advanced target organ damage were to predict failure to respond to RDN then these findings are difficult to rationalise, since our data suggest that those individuals who have started to develop an impairment in (circumferential) cardiac function actually responded to RDN with a greater reduction in BP. Conversely, those individuals with a supra-normal ejection fraction and a lower end-systolic volume (which may indicate a stiffened ventricle with impaired filling a diastolic dysfunction in the context of hypertensive heart disease) were indeed less likely to respond to RDN. Cardiac remodelling in the context of hypertension is complex, and the mechanical interaction between the myocardium and aorta (ventriculo-vascular coupling) further impacts cardiac remodelling and diastolic dysfunction in hypertensive heart disease (Fox and Maurer 2005, Rodrigues, Amadu et al. 2016). These complex interactions will make it unlikely that a single marker of cardiac function or structural remodelling will predict the response to RDN in the individual.

Fengler et al. have looked at predictors of response in profound RDN responders (with a reduction in daytime ABPM of >20 mmHg), and identified younger age, use of ultrasound RDN, combined diuretic therapy and baseline BP (and pulse wave velocity in a subset of patients) as predictors of BP reduction pronounced BP reduction (Fengler, Rommel et al. 2018). Younger age and lower vascular stiffness may indicate reversibility in the vascular changes associated with hypertension, and may represent the opposite end of the spectrum of pathology to those individuals with isolated systolic hypertension and irreversible vascular remodeling in whom RDN has proved to be less successful (Ewen, Ukena et al. 2015, Mahfoud, Bakris et al. 2017). Thiazide and loop diuretics have a sympathoexcitatory effect and RDN may act to counterbalance this adverse physiological effect, whilst patients continue to receive the beneficial effects of sequential nephron blockade (Vink and Blankestijn 2013, Fengler, Rommel et al. 2018, Schlaich, Carnagarin et al. 2018).

5.6.4.1 Limitations

As raised on multiple previous occasions in this manuscript, the lack of complete ABPM outcome data is a major limitation of this study. The use of office SBP as the primary outcome measure has been critiqued within the field, particularly since office BP is likely to be more vulnerable to regression to the mean. If this is indeed the case, then this may explain the finding that those with higher baseline oSBP are more likely to have a reduction in SBP following intervention. Importantly, amongst the patients with available data, there was no change in ambulatory BP following RDN in this study, and no correlation between baseline ABPM parameters and the change in oSBP following the procedure (see Table 5-36). The blood pressure endpoint used matters; Persu et al. demonstrated that when office BP was used as the primary outcome measure following RDN, responders were more likely to have white coat hypertension with no reduction in ABPM following the intervention, however, when response was defined by ABPM outcomes, then baseline ABPM was a predictor of response to treatment (Persu, Azizi et al. 2014).

Once again, our inability to confirm medication adherence and the lack of a standardised medication regime impact data interpretation in this cohort. It would not be surprising to expect that medications, particularly those targeting the sympathovascular axis such as beta-blockers or centrally acting sympatholytic agents, would affect the BP response to RDN (Barber-Chamoux and Esler 2017), and both sympathoinhibitory (e.g. renin-angiotensin-aldosterone system inhibitors and centrally acting sympatholytic drugs such as moxonidine and clonidine) and sympathoexcitatory (e.g. diuretics, vasodilators and calcium channel blockers) medications were prescribed in this study (Vink and Blankestijn 2013).

It might have been expected that patients prescribed sympathoinhibitory drugs would have lower baseline SNA and therefore be less likely to respond the RDN which is hypothesised to work via a sympatholytic effect (Fink and Phelps 2017). However, it appears that antihypertensive drugs which act to reduce SNA may have an additive hypotensive effect with RDN. In Symplicity HTN-3, greater reductions in office BP were seen in participants taking beta-blockers and aldosterone receptors antagonists (Kandzari, Bhatt et al. 2015), and when data from Symplicity HTN-3 were pooled with data from the Global Symplicity Registry the strongest predictors of response to RDN after combined systolic and diastolic hypertension (as opposed to ISH), were the use of an aldosterone antagonist and the non-use of a vasodilator (Mahfoud, Bakris et al. 2017). The use of a centrally acting sympatholytic agent has also been reported to enhance the BP response to RDN (2011, Barber-Chamoux and Esler 2017). We did not observe an interaction between baseline medications and oSBP outcomes in this small cohort, however our subjects were on diverse medications regimes, reflecting prolonged attempts at pharmacological BP management in most of these patients, and adherence could not be formally confirmed. Aldosterone antagonists and vasodilators are known to have a low persistence and compliance (Barber-Chamoux and Esler 2017), and total adherence decreases as the number of prescribed antihypertensive medications increases (Jung, Gechter et al. 2013, Barber-Chamoux and Esler 2017). The indication for different agents must be considered, and the greater BP reduction with RDN amongst patients prescribed aldosterone antagonists may relate to a higher baseline SBP in patients requiring a fourth line agent, as well as the association between aldosterone antagonist prescribing and younger age and a history of significantly more hypertensive crises observed in the Symplicity HTN-3 study cohort (Kandzari, Bhatt et al. 2015).

Ultimately, this was a pilot study to assess the feasibility of assessing a broad autonomic profile in patients with resistant hypertension undergoing RDN and sought to identify signals for predictors of BP response, but the study was not adequately powered to validate these findings.

5.6.5 Conclusions

A higher baseline office SBP and greater baseline spontaneous sympathetic baroreflex gain may be useful markers for identifying patients who would be likely to benefit from RDN. Limitations in these findings, particularly the use of office rather than ambulatory BP measures, must be taken into account and further research is required to confirm these observations in a larger population. Contrary to our hypotheses, markers of

sympathetic nerve activity, arterial stiffness, chemoreflex sensitivity and systemic inflammation were not associated with a BP reduction following RDN in this small cohort. Antihypertensive medications are likely to interact with renal nerve ablation and the use of certain agents prior to RDN may enhance or inhibit any BP response. The interaction between variable medication regimes and medication adherence may well impact BP outcomes and data interpretation and we await the full outcomes of the Spyral ON and OFF-MED studies with great interest (Kandzari, Kario et al. 2016).

6 General discussion

6.1 Study outcomes

This study aimed to initiate the development of indices to predict response to, and aid patient selection for, renal denervation, and to investigate physiological measures which could be used to assess the efficacy of renal denervation for use at the time of the procedure.

6.1.1 Primary study outcomes

6.1.1.1 Predictors of response to renal denervation

The primary aim was to generate data to accurately predict whether a patient with resistant hypertension would respond to renal denervation therapy with a reduction in blood pressure. From univariate analyses, a higher baseline office SBP, higher baseline office MAP and a greater baseline spontaneous sympathetic baroreflex gain were all identified as potentially useful markers for identifying patients who would be likely to benefit from RDN.

Higher office SBP has been reported as a predictor of response to RDN (Bhatt, Kandzari et al. 2014, Vogel, Kirchberger et al. 2014, Bohm, Mahfoud et al. 2015, Kandzari, Bhatt et al. 2015). It has been hypothesised that higher SBP may indicate a greater severity or longer duration of hypertension and thus increased target organ damage and/or vascular stiffness (Barber-Chamoux and Esler 2017). In this study baseline measures of sympathetic nerve activity, aortic distensibility, left ventricular mass and renal function did not predict a response to RDN, and notably there was no correlation between either baseline office pulse pressure or baseline office DBP and the blood pressure reduction following RDN. These results were therefore not in keeping with the previous reports of a poor response to RDN in patients with isolated systolic hypertension (Ewen, Ukena et al. 2015, Mahfoud, Bakris et al. 2017). A concern is that the association between a higher baseline SBP and a greater reduction in SBP following RDN may represent regression to the mean, and the consensus in the field is that ABPM should be used as the primary outcome measure in all future studies of the efficacy of RDN in part to minimise this effect (Persu, Azizi et al. 2014, Barber-Chamoux and Esler 2017).

The evidence for the role of MSNA as a predictor of response to RDN is unclear. In this, and other studies, there was no correlation between the change in MSNA and the change in oSBP following RDN (Hering, Lambert et al. 2013, Grassi, Seravalle et al. 2015). This is the first study to have specifically investigated the use of MSNA in predicting response to RDN; there was no direct correlation between baseline MSNA and the change in office SBP following RDN. However, looking at the individual BP and MSNA responses following RDN (see Figure 5-17), the pattern of response did correlate over the follow-up period in some of the participants, although this was not consistent. Our data have also suggested that the impact of gender on MSNA and sympathovascular transduction must be taken into account in future study paradigms (Hart, Charkoudian et al. 2011, Briant, Burchell et al. 2016).

6.1.1.2 Measures of procedural efficacy

The secondary aim of the study was to develop methods for quantifying the procedural success of renal denervation that could be applied at the time of the procedure to enable accurate interpretation of outcome data, and potentially to guide the delivery of adequate radiofrequency ablation or to aid the development of improved catheter technologies.

Resting renal blood flow, changes in renal blood flow in response to handgrip stress, and the changes in systemic BP in response to intra-renal arterial infusion of adenosine were investigated as potential measures of the procedural effectiveness of RDN. The advantage of all these approaches was that the studies were performed under conscious analgosedation, as opposed to general anaesthesia, making them more amenable to use in the general catheter laboratory. This is in comparison to the assessment of responses to direct electrical stimulation of the renal nerves which constitutes the other major approach to these issues reported in the literature and has been performed under general anaesthesia (Gal, de Jong et al. 2015, Chinushi, Suzuki et al. 2016, de Jong, Adiyaman et al. 2016, de Jong, Hoogerwaard et al. 2018, Hoogerwaard, de Jong et al. 2018).

The measurement of changes in renal blood flow and renal vascular resistance in response to dynamic handgrip stress show potential for this application. From the group data, an inability to increase renal vascular resistance with handgrip after denervation indicated disruption of the renal sympathetic nerves, although the technique may lack sensitivity to guide ablation in every individual patient. Tests to differentiate between afferent and efferent renal nerve function would provide useful mechanistic information underlying any successful anti-hypertensive effect of RDN. Unfortunately, adenosine as trialled in this sub-study, is not an adequate afferent stimulus, and a cocktail of afferent renal nerve stimuli, including agents such as adenosine, low pH and bradykinin, may prove more effective (Katholi, Whitlow et al. 1984, Barry and Johns 2015).

6.1.2 Secondary study outcomes

6.1.2.1 Clinical outcomes

One of the major outstanding questions in the field, particularly in the aftermath of Symplicity HTN-3, has been whether renal denervation is truly effective in lowering BP, and perhaps more importantly, in reducing target organ damage and/or improving mortality and morbidity. The recent publication of the SPYRAL HTN-ON and -OFF MED studies and the RADIANCE HTN-SOLO study (Townsend, Mahfoud et al. 2017, Azizi, Schmieder et al. 2018, Kandzari, Bohm et al. 2018), which reported significant improvements in ABPM outcomes in sham-controlled studies with structured medication regimes (including off medication), has gone some way to reignite enthusiasm for the technique.

This was a pilot study, and as such, was not powered to demonstrate a significant improvement in BP following RDN. Despite this, 61% of study participants responded to RDN with a ≥10 mmHg reduction in office SBP at 6 months post denervation, and whilst

the study failed to achieve a significant reduction in office SBP at 6 months after RDN, there was a significant change in office SBP across the full follow-up period, reaching significance at 12 months post-RDN. Notably, a reduction in SBP was associated with an improvement (reduction) in LV mass, and improvements in peak radial and peak circumferential strain following RDN. The use of office SBP as the primary outcome measure in RDN studies has been heavily criticised, but the reduction in left ventricular mass post-RDN seen in this cohort is a more robust indication of a genuine clinical benefit for study participants. In a recent metanalysis, Kordalis et al. reported positive effects of RDN on left ventricular mass, augmentation index and pulse wave velocity (Kordalis, Tsiachris et al. 2018), and as RDN looks to the future, data evidencing an improvement in cardiovascular mortality and morbidity following the intervention will be required if RDN is to justify a place in mainstream clinical practice.

6.1.2.2 Mechanisms for the antihypertensive effect of renal denervation

Whilst the evidence supporting a clinically relevant antihypertensive effect for RDN is strengthening (Townsend, Mahfoud et al. 2017, Kandzari, Bohm et al. 2018), the mechanisms underlying a reduction in BP following RDN are still to be fully defined. There were reductions in office SBP and LV mass (and LV interstitial fibrosis) over the course of this study, but these changes were independent of any change in MSNA, total peripheral resistance or aortic distensibility. It is notable that MSNA did not correlate with TPR, however, whilst there was no change in MSNA following RDN, RDN responders tended towards a reduction in TPR at 6 months, whereas RDN non-responders had a significant increase in TPR following RDN. The method used to calculate TPR in this study used several assumptions and future projects should employ a more robust technique to quantify changes in vascular tone following RDN.

It is interesting to note that a significant reduction in office SBP was not attained until 12 months after the procedure. This observation is similar to the sustained and progressive reduction in SBP reported in earlier studies including Symplicity HTN-1 (Krum, Schlaich et al. 2014). Considering this timescale, RDN is likely to have an affect beyond an acute reduction in sympathetic nerve activity and vascular tone, and may act through vascular remodelling and changes in vascular stiffness, gradual resetting of the baroreflex or the sensitivity of sympathovascular transduction, or slow shifts in the balance of the renin-angiotensin-aldosterone system (Krum, Schlaich et al. 2014). Data from this study do not back any one of these mechanisms, which may relate to the small cohort size, differences in SNA and sympathovascular transduction between subjects of varying age and gender, a lack of sensitivity and specificity in the use of MSNA as a surrogate for renal SNA, or factors such as a change in the operating point (rather than the sensitivity) of the baroreflex to act over a lower BP range which we have not quantified, and where further research is required. We had hoped to be able to shed some light on the relative impact of afferent or efferent renal nerve disruption by looking at the relative changes in the afferent and efferent physiological procedural measures of efficacy, but these data were not sufficiently well defined to draw any further conclusions. It is also unlikely that the afferent and efferent nerves can be differentially ablated because they are anatomically juxtaposed (Sakakura, Ladich et al. 2014, Kopp 2015), despite attempts at this using renal nerve stimulation guided ablation (Fudim, Sobotka et al. 2018).

6.2 Project critique

6.2.1 Pilot study

This pilot study successfully demonstrated the feasibility of assessing a broad autonomic profile in patients with treatment resistant hypertension. Each study visit lasted 3-4 hours and participants were happy to attend for repeated physiological testing, including microneurography, baroreflex assessment using the modified Oxford technique and spirometry for chemoreflex analyses. We were able to demonstrate good inter- and intra-individual variability for measurement of MSNA (see Section 4.3.6.4) and as a group have established a substantial dataset, including participants from this study, quantifying target organ damage including hypertensive heart disease using magnetic resonance imaging (Burchell, Rodrigues et al. 2017). As such, we are well placed to apply these techniques to further studies in the field. We have also been able to confirm the excellent safety profile for endovascular, radiofrequency, renal nerve ablation, as reported in the wider literature (Bhatt, Kandzari et al. 2014, Bohm, Mahfoud et al. 2015, Burchell, Chan et al. 2016).

6.2.2 Limitations

As a pilot study, this project was not powered to demonstrate a significant reduction in BP following RDN, but this issue was further compounded by difficulties in recruitment. We had ethical approval to recruit up to thirty participants but were only able to enrol 19 subjects (with one early withdrawal). Thorough assessment in the Bristol Heart Institute specialist Hypertension Clinic identified patients meeting exclusion criteria due to white coat effect, poor medication adherence or multiple intolerances, and ineligible renal anatomy. Specialist review of patients' antihypertensive regimes, including the increased use of aldosterone receptor antagonists, also optimised BP control resulting in a good outcome for the patients, but rendering many potential participants ineligible for the study.

The study protocol was devised to build on the findings of Symplicity HTN-2 (Esler, Krum et al. 2010), and prior to the critique of Symplicity HTN-3 (Bhatt, Kandzari et al. 2014), and will therefore be subject to similar criticisms to these earlier studies, including incomplete ABPM data and no objective confirmation of medication adherence (Esler 2014, Kandzari, Bhatt et al. 2015). Since completion of the study follow-up visits we have be able to obtain ethical approval and retrospective consent to measure antihypertensive drug metabolites in frozen urine samples from five participants. These data are shown in Appendix 5, but to summarise, whilst there was no change in the percentage of prescribed medications detected over the course of the study, adherence levels ranged from 0-100%. Adherence (or non-adherence) was reasonable stable within the individual, but the huge variability in inter-individual adherence and the differing medication regimes prescribed limit our ability to interpret outcome data.

6.3 Future directions

As we face a resurgence in interest in the field of renal denervation, the full results of largescale, sham-controlled clinical trials including the SPYRAL HTN studies, will

hopefully provide definitive evidence of the antihypertensive effect of RDN and the level of blood pressure reduction that may be expected. Industry will also continue to drive forward the development of second and third generation catheter technologies and ablation modalities, and anatomical (or stimulation led) targeting of the renal nerve ablations will be optimised. Once the technical aspects of the procedure have been refined the physiological questions regarding the mechanisms underlying RDN, predictors of response and measures of procedural efficacy should be revisited. Future studies must use robust endpoints based on ambulatory BP monitoring and reversal of target organ damage in a clearly defined study population on a standardized and objectively assessed medication regime. If RDN can be proven to have a positive sympathomodulatory effect, then future research should investigate a role for the intervention in other sympathetically mediated conditions such as refractory arrhythmias, sleep apnoea and heart failure.

Renal denervation must walk before it can run and has been a lesson in the importance of careful study design and caution before the larger scale roll-out of a novel technology. The clinical challenge that RDN aims to address remains a valid problem in the face of a global epidemic of cardiovascular disease and increasing evidence demonstrating poor patient adherence with conventional pharmacological approach to managing hypertension (2017). It remains to establish which individuals will benefit from this invasive and evolving interventional approach to hypertension.

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8 Appendices

8.1 Appendix 1. Prescribed medications

Table shows individual patient medications at each study time point. Whole dose equivalents (WDE) have be calculated to aid comparison between different drugs and drugs classes. ACE; angiotensin converting enzyme inhibitor, ARB; angiotensin receptor blocker, RI; renin inhibitor, CCB; calcium channel blocker, MRA; mineralocorticoid antagonist.

		Baseline			1 month			3 months			6 months			12 Months		
		Drug	**Dose**	**WDE**	**Drug**	**Dose**	**WDE**	**Drug**	**Dose**	**WDE**	**Drug**	**Dose**	**WDE**	**Drug**	**Dose**	**WDE**
1	ACE/ARB/RI	Lisinopril	20	0.25	Lisinopril	20	0.25	Lisinopril	20	0.25	Lisinopril	20	0.25	Lisinopril	80	1
	CCB	Tildiem	300	0.75	Tildiem	300	0.75	Tildiem	300	0.75	Tildiem	300	0.75	Tildiem	300	0.75
	Diuretic		0	0		0	0		0	0		0	0		0	0
	MRA		0	0		0	0		0	0		0	0	Spironolactone	25	0.25
	β-Blocker		0	0		0	0		0	0		0	0		0	0
	α-Blocker	Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	16	1
	Centrally Acting		0	0		0	0		0	0		0	0		0	0
	Vasodilator	ISMN	20	0.17	ISMN	20	0.17	ISMN	20	0.17	ISMN	20	0.17	ISMN	25	0.21
	Other		0	0		0	0		0	0		0	0		0	0
	No Drugs			**4**			**4**			**4**			**4**			**5**
	No classes			**4**			**4**			**4**			**4**			**5**
	WDE			**1.7**			**1.7**			**1.7**			**1.7**			**3.2**
2	ACE/ARB/RI	Losartan	25	0.25	Losartan	25	0.25	Losartan	25	0.25	Losartan	25	0.25	Losartan	25	0.25
	CCB		0	0		0	0		0	0		0	0		0	0
	Diuretic	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1
	MRA		0	0		0	0		0	0		0	0		0	0
	β-Blocker		0	0		0	0		0	0		0	0		0	0
	α-Blocker		0	0		0	0		0	0		0	0		0	0
	Centrally Acting	Moxonidine	400	0.67	Moxonidine	600	1	Moxonidine	600	1	Moxonidine	600	1	Moxonidine	600	1
	Vasodilator		0	0		0	0		0	0		0	0		0	0
	Other		0	0		0	0		0	0		0	0		0	0
	No Drugs			**3**			**3**			**3**			**3**			**3**
	No classes			**3**			**3**			**3**			**3**			**3**
	WDE			**1.9**			**2.3**			**2.3**			**2.3**			**2.3**
3	ACE/ARB/RI	Lisinopril	20	0.25	Lisinopril	20	0.25	Lisinopril	20	0.25	Lisinopril	20	0.25	Lisinopril	20	0.25
	CCB	Felodipine	10	1	Felodipine	10	1	Felodipine	10	1	Felodipine	10	1	Felodipine	10	1

	Diuretic	Furosemide	40	0.5	Furosemide	40	0.5	Furosemide	40	0.5	Furosemide	40	0.5	Furosemide	40	0.5
	MRA	Spironolactone	200	2	Spironolactone	200	2	Spironolactone	200	2	Spironolactone	200	2	Spironolactone	200	2
	β-Blocker	Bisoprolol	20	2	Bisoprolol	20	2	Bisoprolol	20	2			0			0
	α-Blocker	Doxazosin	16	1	Doxazosin	16	1	Doxazosin	16	1	Doxazosin	16	1	Doxazosin	16	1
	Centrally Acting		0	0		0	0		0	0		0	0		0	0
	Vasodilator		0	0		0	0		0	0		0	0		0	0
	Other	Candesartan	32	1	Candesartan	32	1	Candesartan	32	1	Candesartan	32	1	Candesartan	32	1
	Other	Aliskiren	150	0.5	Aliskiren	150	0.5	Aliskiren	150	0.5	Aliskiren	150	0.5	Aliskiren	150	0.5
	No Drugs			**8**			**8**			**8**			**7**			**7**
	No classes			**6**			**6**			**6**			**5**			**5**
	WDE			**8.3**			**8.3**			**8.3**			**6.3**			**6.3**
4	ACE/ARB/RI	Perinodpril	4	0.5	Perinodpril	4	0.5				Perinodpril	4	0.5		0	0
	CCB		0	0		0	0					0	0		0	0
	Diuretic	BFZ	2.5	1	BFZ	2.5	1				BFZ	2.5	1		0	0
	MRA		0	0		0	0					0	0		0	0
	β-Blocker	Labetolol	800	1			0				Labetolol	200	0.25	Labetolol	200	0.25
	α-Blocker		0	0			0					0	0		0	0
	Centrally Acting	Moxonidine	200	0.33			0					0	0		0	0
	Vasodilator		0	0			0					0	0		0	0
	Other	Aliskiren	150	0.5			0					0	0		0	0
	No Drugs			**5**			**2**						**3**			**1**
	No classes			**4**			**2**						**3**			**1**
	WDE			**3.3**			**1.5**						**1.8**			**0.3**
5	ACE/ARB/RI	Candesartan	32	1	Candesartan	32	1	Candesartan	32	1	Candesartan	32	1	Candesartan	32	1
	CCB	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1
	Diuretic	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1
	MRA	Spironolactone	25	0.25		0	0		0	0		0	0		0	0
	β-Blocker	Atenolol	100	1		0	0		0	0		0	0		0	0
	α-Blocker	Doxazosin	8	0.5		0	0		0	0		0	0		0	0
	Centrally Acting		0	0		0	0		0	0		0	0		0	0

	Vasodilator	ISMN	80	0.67	0	0	0	0	0	0		0	0
	Other		0	0	0	0	0	0	0	0		0	0
	No Drugs			**7**		**3**		**3**		**3**			**3**
	No classes			**7**		**3**		**3**		**3**			**3**
	WDE			**5.4**		**3.0**		**3.0**		**3.0**			**3.0**
6	ACE/ARB/RI	Ramipril	10	1	Ramipril	10	1	Ramipril	10	1	Ramipril	10	1	Ramipril	10	1
	CCB	Felodipine	10	1	Felodipine	10	1	Felodipine	10	1	Felodipine	10	1	Felodipine	10	1
	Diuretic	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1
	MRA	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25
	β-Blocker	Bisoprolol	10	0.5	Bisoprolol	10	0.5	Bisoprolol	10	0.5	Bisoprolol	10	0.5	Bisoprolol	10	0.5
	α-Blocker		0	0		0	0		0	0		0	0		0	0
	Centrally Acting		0	0		0	0		0	0		0	0		0	0
	Vasodilator		0	0		0	0		0	0		0	0		0	0
	Other		0	0		0	0		0	0		0	0		0	0
	No Drugs			**5**		**5**		**5**		**5**			**5**
	No classes			**5**		**5**		**5**		**5**			**5**
	WDE			**3.8**		**3.8**		**3.8**		**3.8**			**3.8**
7	ACE/ARB/RI	Ramipril	10	1	Ramipril	10	1	Ramipril	10	1	Ramipril	10	1	Ramipril	10	1
	CCB	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1
	Diuretic	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1
	MRA	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25			0	Spironolactone	25	0.25
	β-Blocker		0	0		0	0		0	0		0	0		0	0
	α-Blocker	Doxazosin	4	0.25	Doxazosin	4	0.25	Doxazosin	4	0.25	Doxazosin	4	0.25	Doxazosin	2	0.13
	Centrally Acting		0	0		0	0		0	0		0	0		0	0
	Vasodilator		0	0		0	0		0	0		0	0		0	0
	Other		0	0		0	0		0	0		0	0		0	0
	No Drugs			**5**		**5**		**5**		**4**			**5**
	No classes			**5**		**5**		**5**		**4**			**5**
	WDE			**3.5**		**3.5**		**3.5**		**3.3**			**3.4**

8	ACE/ARB/RI	Perindopril	8	1	Perindopril	8	1	Perindopril	8	1	Candesartan	32	1	Candesartan	32	1
	CCB	Lacidipine	10	1.67	Lacidipine	10	1.67	Lacidipine	10	1.67	Lacidipine	10	1.67	Lacidipine	10	1.67
	Diuretic		0	0		0	0		0	0		0	0		0	
	MRA	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25
	β-Blocker	Celiprolol	400	1	Celiprolol	400	1	Celiprolol	400	1	Celiprolol	400	1	Celiprolol	400	1
	α-Blocker	Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	12	0.75
	Centrally Acting		0	0		0	0		0	0		0	0		0	
	Vasodilator		0	0		0	0		0	0						
	Other	Aliskiren	150	0.5	Aliskiren	150	0.5	Aliskiren	150	0.5				Aliskiren	150	0.5
	Other	Candesartan	32	1	Candesartan	32	1	Candesartan	32	1					0	
	No Drugs			**7**			**7**			**7**			**5**			**6**
	No classes			**5**			**5**			**5**			**5**			**5**
	WDE			**5.9**			**5.9**			**5.9**			**4.4**			**5.2**
9	ACE/ARB/RI	Lisinopril	5	0.06		0	0		0	0		0	0			
	CCB		0	0		0	0		0	0		0	0			
	Diuretic		0	0		0	0		0	0		0	0			
	MRA		0	0		0	0		0	0		0	0			
	β-Blocker		0	0	Bisoprolol	2.5	0.25		0	0		0	0			
	α-Blocker	Indoramin	25	0.12 5		0	0		0	0		0	0			
	Centrally Acting		0	0		0	0		0	0		0	0			
	Vasodilator		0	0		0	0		0	0		0	0			
	Other	Aliskiren	300	1		0	0		0	0		0	0			
	No Drugs			**3**			**1**			**0**			**0**			
	No classes			**2**			**1**			**0**			**0**			
	WDE			**1.2**			**0.3**			**0.0**			**0.0**			
10	ACE/ARB/RI	Losartan	100	1	Losartan	50	0.5	Losartan	100	1	Losartan	100	1	Losartan	50	0.5
	CCB	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1
	Diuretic	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1
	MRA	Spironolactone	50	0.5	Spironolactone	75	0.75	Spironolactone	75	0.75	Spironolactone	75	0.75	Spironolactone	75	0.75
	β-Blocker	Atenolol	100	1	Bisoprolol	1.25	0.13	Bisoprolol	2.5	0.25	Bisoprolol	10	1	Bisoprolol	15	1.5

	α-Blocker	Doxazosin	16	1		0	0		0	0		0	0		0	0
	Centrally Acting		0	0		0	0		0	0		0	0		0	0
	Vasodilator		0	0		0	0		0	0		0	0	ISMN	30	0.25
	Other		0	0		0	0		0	0	Aliskiren	150	0.5	Aliskiren	150	0.5
	No Drugs			**6**			**5**			**5**			**6**			**7**
	No classes			**6**			**5**			**5**			**5**			**6**
	WDE			**5.5**			**3.4**			**4.0**			**5.3**			**5.5**
11	ACE/ARB/RI	Ramipril	10	1	Ramipril	10	1	Ramipril	10	1	Ramipril	10	1	Ramipril	10	1
	CCB	Adalat LA (nifedipine)	60	0.67	Adalat LA (nifedipine)	60	0.67		0	0	Adalat LA (nifedipine)	60	0.67	Adalat LA (nifedipine)	60	0.67
	Diuretic	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1
	MRA	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25
	β-Blocker	Bisoprolol	10	1	Bisoprolol	10	1	Bisoprolol	10	1	Bisoprolol	10	1	Bisoprolol	10	1
	α-Blocker		0	0		0	0	Doxazosin	4	0.25	Doxazosin	4	0.25	Doxazosin	16	1
	Centrally Acting	Moxonidine	200	0.33	Moxonidine	200	0.33	Moxonidine	200	0.33	Moxonidine	200	0.33	Moxonidine	600	1
	Vasodilator		0	0		0	0		0	0		0	0		0	0
	Other		0	0		0	0		0	0		0	0		0	0
	No Drugs			**6**			**6**			**6**			**7**			**7**
	No classes			**6**			**6**			**6**			**7**			**7**
	WDE			**4.3**			**4.3**			**3.8**			**4.5**			**5.9**
12	ACE/ARB/RI	Enalapril	40	1	Enalapril	40	1	Enalapril	40	1	Enalapril	40	1	Enalapril	40	1
	CCB		0	0		0	0		0	0		0	0		0	0
	Diuretic	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1
	MRA		0	0		0	0		0	0		0	0		0	0
	β-Blocker		0	0		0	0		0	0		0	0		0	0
	α-Blocker		0	0		0	0		0	0		0	0		0	0
	Centrally Acting		0	0		0	0		0	0		0	0		0	0
	Vasodilator		0	0		0	0		0	0		0	0		0	0
	Other	Irbesartan	150	0.5	Irbesartan	150	0.5	Irbesartan	150	0.5	Irbesartan	150	0.5	Irbesartan	150	0.5
	No Drugs			**3**			**3**			**3**			**3**			**3**
	No classes			**2**			**2**			**2**			**2**			**2**

	WDE			**2.5**			**2.5**			**2.5**			**2.5**			**2.5**
13	ACE/ARB/RI	Candesartan	4	0.13		0	0					0	0	Losartan	50	0.5
	CCB	Amlodipine	20	2	Amlodipine	20	2				Amlodipine	10	1	Amlodipine	10	1
	Diuretic	Furosemide	20	0.25	Furosemide	20	0.25				Furosemide	20	0.25	Furosemide	20	0.25
	MRA	Spironolactone	50	0.5	Spironolactone	50	0.5				Spironolactone	50	0.5	Spironolactone	50	0.5
	β-Blocker	Labetolol	600	0.75	Labetolol	600	0.75				Labetolol	600	0.75	Labetalol	600	0.75
	α-Blocker	Doxazosin	16	1	Doxazosin	12	0.75				Doxazosin	8	0.5		0	0
	Centrally Acting		0	0		0	0					0	0		0	0
	Vasodilator	Tadalafil	2.5	0.06	Tadalafil	2.5	0.06				Tadalafil	2.5	0.06	Tadalafil	2.5	0.06
	Other		0	0		0	0					0	0		0	0
	No Drugs			**7**			**6**						**6**			**6**
	No classes			**7**			**6**						**6**			**6**
	WDE			**4.7**			**4.3**						**3.1**			**3.1**
14	ACE/ARB/RI	Perinodpril	8	1	Perinodpril	8	1	Perinodpril	8	1	Perinodpril	8	1	Perinodpril	8	1
	CCB	Nifedipine	30	0.33	Nifedipine	30	0.33	Nifedipine	30	0.33	Nifedipine	30	0.33	Nifedipine	30	0.33
	Diuretic		0	0		0	0		0	0		0	0		0	0
	MRA		0	0		0	0		0	0		0	0		0	0
	β-Blocker	Bisoprolol	1.25	0.13	Bisoprolol	1.25	0.13	Bisoprolol	1.25	0.13		0	0	Carvedilol	6.25	0.13
	α-Blocker		0	0		0	0		0	0		0	0		0	0
	Centrally Acting	Moxonidine	600	1	Moxonidine	600	1	Moxonidine	600	1	Moxonidine	600	1	Moxonidine	600	1
	Vasodilator		0	0		0	0		0	0		0	0		0	0
	Other		0	0		0	0		0	0		0	0		0	0
	No Drugs			**4**			**4**			**4**			**3**			**4**
	No classes			**4**			**4**			**4**			**3**			**4**
	WDE			**2.5**			**2.5**			**2.5**			**2.3**			**2.5**
15	ACE/ARB/RI	Ramipril	10	1				Ramipril	10	1	Ramipril	10	1	Ramipril	10	1
	CCB	Amlodipine	10	1				Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1
	Diuretic	BFZ	2.5	1				BFZ	2.5	1	BFZ	2.5	1	BFZ	2.5	1
	MRA		0	0					0	0		0	0		0	0

	β-Blocker	Atenolol	100	1			Atenolol	100	1	Atenolol	100	1	Atenolol	100	1
	α-Blocker	Doxazosin	8	0.5			Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	8	0.5
	Centrally Acting	Moxonidine	800	1.33				0	0	Moxonidine	400	0.67	Moxonidine	400	0.67
	Vasodilator		0	0				0	0		0	0		0	0
	Other		0	0				0	0		0	0		0	0
	No Drugs			**6**					**5**			**6**			**6**
	No classes			**6**					**5**			**6**			**6**
	WDE			**5.8**					**4.5**			**5.2**			**5.2**
16	ACE/ARB/RI	Candesartan	2	0.06	Candesartan	2	0.06	Candesartan	2	0.06	Candesartan	2	0.06	Candesartan	2	0.06
	CCB		0	0		0	0		0	0		0	0		0	
	Diuretic		0	0		0	0		0	0		0	0		0	
	MRA		0	0		0	0		0	0		0	0		0	
	β-Blocker		0	0		0	0		0	0		0	0		0	
	α-Blocker	Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	8	0.5	Doxazosin	4	0.25
	Centrally Acting	Moxonidine	100	0.17	Moxonidine	100	0.17	Moxonidine	100	0.17	Moxonidine	100	0.17	Monoxidine	200	0.33
	Vasodilator		0	0		0	0		0	0	Tadalafil	2.5	0.06		0	
	Other		0	0		0	0		0	0		0	0	Ranolazine	750	0.5
	No Drugs			**3**			**3**			**3**			**4**			**4**
	No classes			**3**			**3**			**3**			**4**			**4**
	WDE			**0.7**			**0.7**			**0.7**			**0.8**			**1.1**
17	ACE/ARB/RI	Perindopril	8	1	Perindopril	4	0.5	Perindopril	8	1	Perindopril	8	1	Perindopril	8	1
	CCB	Amlodipine	10	1	Amlodipine	5	0.5	Amlodipine	10	1	Amlodipine	10	1	Amlodipine	10	1
	Diuretic	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1
	MRA	Epleronone	100	2		0	0		0	0		0	0		0	0
	β-Blocker	Atenolol	100	1	Atenolol	50	0.5	Labetolol	800	1	Labetolol	800	1	Labetalol	480	0.6
	α-Blocker	Doxazosin	16	1		0	0	Doxazosin	8	0.5	Doxazosin	16	1	Doxazosin	16	1
	Centrally Acting	Moxonidine	600	1		0	0		0	0	Tadalafil	2.5	1	Clonidine	200	0.17
	Vasodilator	Hydralazine	150	1.5		0	0		0	0	Hydralazine	150	1.5	Hydralazine	150	1.5
	Other		0	0		0	0	Amiloride	5	0.25	Amiloride	10	0.5		0	
	No Drugs			**8**			**4**			**6**			**8**			**7**

	No classes			**8**			**4**			**6**			**8**			**7**
	WDE			**9.5**			**2.5**			**4.75**			**8**			**6.3**
18	ACE/ARB/RI	Losartan	100	1	Losartan	100	1	Losartan	100	1	Losartan	100	1	Losartan	100	1
	CCB		0			0			0			0			0	
	Diuretic	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	2.5	1	Indapamide	1.5	1	Indapamide	1.5	1
	MRA	Spironolactone	12.5	0.13	Spironolactone	12.5	0.13	Spironolactone	25	0.25	Spironolactone	25	0.25	Spironolactone	25	0.25
	β-Blocker		0			0			0			0			0	
	α-Blocker		0			0			0			0			0	
	Centrally Acting					0			0			0			0	
	Vasodilator		0			0			0			0			0	
	Other		0			0			0			0			0	
	No Drugs			**3**			**3**			**3**			**3**			**3**
	No classes			**3**			**3**			**3**			**3**			**3**
	WDE			**2.1**			**2.1**			**2.3**			**2.3**			**2.3**

8.2 Appendix 2. Correlations between autonomic parameters at baseline

For each correlation data for shown for the number of patients (n), the Pearson's or Spearman's correlation coefficient (R), and the p valve, with $p<0.05$ taken as significant. Abbreviations: oSBP; office systolic blood pressure, iLVM; left ventricular mass indexed to body surface area, MSNA; muscle sympathetic nerve activity, LF/HF; ratio between heart rate variability low frequency and high frequency spectral power, ssBRST; spontaneous sympathetic vascular baroreflex sensitivity assessed by the threshold method, ssBRSA; spontaneous sympathetic vascular baroreflex sensitivity assessed by the area method, scBRS; spontaneous cardiac baroreflex sensitivity, BEI: baroreflex effectiveness index, HVR; hypoxic ventilatory response overall data for both intermittent and stepped hypoxia methods, HVR int; hypoxic ventilatory response as assessed by the intermittent hypoxia method, HVR step; hypoxic ventilatory response as assessed by the stepped hypoxia method, total CBF; total cerebral blood flow, total CBF %of CO; total cerebral blood flow as a percentage of cardiac output, CRP; C-reactive protein, IL; interleukin, MPO; myeloperoxidase, TNFα; tumour necrosis factor alpha.

Baseline parameter	oSBP			iLVM			Aortic distensibility			MSNA incidence			LF/HF			Transduction		
	n	R	p	n	R	p	n	R	P	n	R	p	n	R	P	n	R	P
ssBRST overall	13	-0.23	0.44	13	-0.65	0.02	10	0.49	0.15	13	0.01	0.96	13	0.05	0.86			
ssBRST rising	13	0.10	0.75	13	0.02	0.96	10	0.20	0.58	13	-0.09	0.77	13	0.03	0.92			
ssBRST falling	13	-0.63	0.02	13	-0.02	0.95	10	-0.03	0.92	13	0.36	0.23	13	-0.10	0.74			
ssBRSA overall	13	-0.53	0.06	13	-0.38	0.20	10	0.52	0.12	13	0.21	0.50	13	0.05	0.88			
ssBRSA rising	13	0.08	0.80	13	-0.31	0.31	10	0.42	0.22	13	-0.11	0.71	13	0.28	0.35			
ssBRSA falling	13	-0.58	0.04	13	0.15	0.63	10	-0.11	0.77	13	0.48	0.09	13	-0.02	0.95			
psBRSAoverall	5	-0.90	0.04	5	0.39	0.52	5	0.32	0.60	5	0.90	0.04	5	0.05	0.94			
psBRSA rising	5	-0.48	0.41	-	-	-	-	-	-	5	0.18	0.77	-	-	-			
psBRSAfalling	5	-0.91	0.03	-	-	-	-	-	-	5	0.90	0.04	-	-	-			
scBRS overall	13	-0.46	0.11	13	-0.39	0.19	11	-0.14	0.68	10	0.42	0.22	13	0.24	0.43			
scBRS rising	13	-0.11	0.71	13	-0.07	0.83	11	0.17	0.61	10	0.21	0.56	13	0.42	0.16			
scBRS falling	11	-0.60	0.05	11	-0.58	0.06	9	-0.39	0.30	8	0.23	0.59	11	0.18	0.60			
BEI overall	16	0.28	0.30	16	0.30	0.27	14	-0.18	0.54	13	-0.06	0.84	16	0.19	0.49			
BEI rising	16	0.41	0.12	16	0.39	0.14	14	-0.20	0.48	13	-0.39	0.19	16	-0.17	0.53			
BEI falling	16	-0.05	0.86	16	0.06	0.82	14	-0.16	0.59	13	0.37	0.22	16	0.24	0.37			
pcBRS overall	10	-0.25	0.49	10	-0.20	0.58	10	0.50	0.14	7	-0.29	0.56	10	0.60	0.07			
Transduction	13	0.31	0.31	13	-0.13	0.65	10	0.09	0.81	13	0.09	0.77	13	-0.13	0.67			
HVR	16	0.02	0.94	16	-0.45	0.08	14	-0.51	0.06	13	0.21	0.49	16	-0.07	0.80	12	0.23	0.48
HVR int	10	-0.29	0.41	10	-0.32	0.37	8	-0.76	0.03	9	0.12	0.75	10	0.23	0.51	9	0.36	0.34
HVR step	6	0.59	0.22	6	-0.62	0.19	6	0.54	0.30	4	-	-	6	-0.31	0.56	3	-	-
Total CBF	16	0.16	0.56	16	0.36	0.17	15	0.17	0.55	12	-0.17	0.59	15	-0.16	0.57	11	0.24	0.48
CBF % of CO	15	-0.25	0.36	15	0.54	0.04	14	0.07	0.81	14	-0.23	0.43	14	-0.07	0.81	11	-0.01	0.97
CRP	9	0.35	0.36	9	-0.62	0.07	9	0.12	0.76	6	0.39	0.45	8	0.06	0.88	5	0.75	0.15
IL-6	9	-0.46	0.22	9	-0.04	0.92	9	0.30	0.43	7	0.27	0.56	9	0.33	0.38	6	0.03	0.96
IL-8	9	-0.20	0.61	9	0.03	0.95	9	0.73	0.03	7	0.21	0.66	9	0.27	0.49	6	-0.09	0.92
IL-10	9	-0.17	0.66	9	0.02	0.95	9	0.30	0.43	7	0.12	0.79	9	0.26	0.49	6	-0.51	0.31
IL-17	9	-0.55	0.13	9	-0.10	0.80	9	-0.02	0.96	7	0.36	0.42	9	0.13	0.74	6	0.00	1.00
MPO	9	-0.49	0.18	9	-0.42	0.26	9	0.58	0.10	7	0.57	0.18	9	0.38	0.31	6	-0.01	0.99
TNF-α	9	-0.17	0.66	9	0.02	0.95	9	0.30	0.43	7	0.12	0.79	9	0.26	0.49	6	-0.51	0.31

Baseline parameter	ssBRST overall			ssBRSA overall			scBRS overall			BEI overall			HVR			HVR int			HVR step		
	n	R	p	n	R	P	n	R	P	n	R	p	n	R	p	n	R	p	n	R	P
ssBRST overall				13	0.84	0.0003															
psBRSA overall	5	-0.52	0.37	5	-0.17	0.78															
scBRS overall	10	0.16	0.66	10	-0.06	0.88															
scBRS rising	10	0.16	<0.05	10	0.30	<0.05															
scBRS falling	8	-0.03	0.94	8	0.08	0.85															
BEI overall	12	-0.47	0.12	12	-0.38	0.22															
BEI rising	12	0.08	0.80	12	-0.07	0.82															
BEI falling	12	-0.07	0.82	12	0.12	0.71															
pcBRS overall	6	-0.03	1.00	6	-0.03	1.00	7	0.89	0.01												
Transduction	13	0.12	0.69	13	0.008	0.98	12	0.007	0.98	12	0.31	0.32									
HVR	12	0.19	0.56	12	0.10	0.76	15	0.22	0.42	15	0.05	0.86									
HVR int	9	-0.02	0.97	9	-0.11	0.78	9	0.13	0.75	9	0.24	0.54									
HVR step	3	-	-	3	-	-	6	-0.09	0.92	6	0.12	0.80									
Total CBF	11	0.38	0.25	11	0.08	0.80	15	-0.48	0.07	15	0.008	0.98	15	-0.07	0.80	9	0.01	0.97	6	0.25	0.63
CBF % of CO	11	0.14	0.67	11	0.24	0.47	14	-0.38	0.18	14	-0.15	0.60	14	-0.27	0.34	9	-0.14	0.72	5	-0.58	0.31
CRP	5	0.24	0.70	5	0.30	0.63	8	0.30	0.47	8	0.20	0.63	8	0.74	0.04	3	-	-	5	0.66	0.22
IL-6	6	0.19	0.72	6	-0.03	0.95	9	0.15	0.70	9	0.01	0.98	9	-0.21	0.58	4	-	-	5	0.21	0.74
IL-8	6	-0.20	0.71	6	0.14	0.80	9	0.30	0.44	9	0.22	0.57	9	-0.45	0.23	4	-	-	5	0.10	0.95
IL-10	6	0.25	0.64	6	0.08	0.89	9	0.19	0.63	9	0.37	0.33	9	-0.02	0.97	4	-	-	5	0.52	0.37
IL-17	6	0.12	0.82	6	-0.04	0.93	9	0.26	0.49	9	-0.05	0.89	9	0.10	0.80	4	-	-	5	0.22	0.72
MPO	6	0.37	0.47	6	0.55	0.26	9	0.86	0.003	9	-0.55	0.12	9	-0.29	0.44	4	-	-	5	0.04	0.95
TNF-α	6	0.25	0.64	6	0.08	0.89	9	0.19	0.63	9	0.37	0.33	9	-0.02	0.97	4	-	-	5	0.52	0.37

8.3 Appendix 3. Correlations between the changes in autonomic parameters at 6 months after renal denervation

For each correlation data for shown for the number of patients (n), the Pearson's or Spearman's correlation coefficient (R), and the p valve, with $p<0.05$ taken as significant. Abbreviations: oSBP; office systolic blood pressure, iLVM; left ventricular mass indexed to body surface area, MSNA; muscle sympathetic nerve activity, LF/HF; ratio between heart rate variability low frequency and high frequency spectral power, ssBRST; spontaneous sympathetic vascular baroreflex sensitivity assessed by the threshold method, ssBRSA; spontaneous sympathetic vascular baroreflex sensitivity assessed by the area method, scBRS; spontaneous cardiac baroreflex sensitivity, BEI: baroreflex effectiveness index, HVR; hypoxic ventilatory response overall data for both intermittent and stepped hypoxia methods, HVR int; hypoxic ventilatory response as assessed by the intermittent hypoxia method, HVR step; hypoxic ventilatory response as assessed by the stepped hypoxia method, total CBF; total cerebral blood flow, total CBF %of CO; total cerebral blood flow as a percentage of cardiac output, CRP; C-reactive protein, IL; interleukin, MPO; myeloperoxidase, TNFα; tumour necrosis factor alpha

Baseline parameter	oSBP			iLVM			Aortic distensibility			MSNA incidence			LF/HF			Transduction		
	n	R	P	n	R	P	n	R	P	n	R	p	N	R	P	n	R	P
ssBRST overall	13	0.03	0.93	13	-0.31	0.30	10	0.24	0.51	10	-0.06	0.87	12	-0.29	0.37			
ssBRST rising	13	0.18	0.55	13	-0.08	0.78	10	-0.10	0.77	10	0.13	0.72	12	-0.06	0.87			
ssBRST falling	13	-0.22	0.47	13	-0.21	0.49	10	0.00	0.99	10	0.19	0.60	12	0.00	>0.9999			
ssBRSA overall	13	0.22	0.47	13	-0.08	0.81	10	0.11	0.76	10	-0.13	0.72	12	0.04	0.90			
ssBRSA rising	13	0.39	0.19	13	0.08	0.79	10	-0.02	0.97	10	-0.20	0.58	12	-0.15	0.64			
ssBRSA falling	13	-0.13	0.68	13	-0.11	0.72	10	-0.24	0.50	10	0.41	0.24	12	0.24	0.44			
psBRSAoverall	5	0.1	0.95	5	0.1	0.95	5	0.3	0.68	5	0.1	0.95	5	0.4	0.52			
scBRS overall	7	-0.20	0.67	11	0.008	0.99	5	-0.32	0.60	5	-0.05	0.93	7	-0.88	0.02			
scBRS rising	7	0.22	0.64	7	0.47	0.28	5	-0.36	0.55	5	-0.27	0.66	7	-0.79	0.048			
scBRS falling	4	-0.26	0.74	4	-	-	4	-	-	4	-	-	4	-	-			
BEI overall	14	-0.08	0.79	14	0.29	0.31	12	-0.43	0.16	9	0.19	0.62	13	0.70	0.008			
BEI rising	14	-0.18	0.54	14	0.29	0.31	12	-0.32	0.31	9	0.21	0.58	13	0.32	0.29			
BEI falling	14	-0.01	0.98	14	0.21	0.46	12	-0.45	0.14	9	0.15	0.70	13	0.87	0.0001			
pcBRS overall	8	-0.12	0.79	8	0.24	0.58	8	0.02	0.98	4	-	-	8	-0.05	0.93			
Transduction	10	0.33	0.35	10	0.17	0.64	8	0.02	0.98	10	-0.14	0.70	10	0.10	0.78			
HVR overall	12	0.24	0.45	12	0.39	0.21	10	-0.72	0.02	8	0.12	0.77	12	0.52	0.08	7	0.40	0.37
HVR intermittent	8	0.04	0.91	8	0.47	0.23	6	-0.95	0.004	5	0.30	0.62	7	0.78	0.04	5	0.65	0.23
HVR stepped	4	-	-	4	-	-	4	-	-	3	-	-	5	-0.55	0.34	2	-	-
Total CBF	14	0.26	0.37	14	0.40	0.16	13	-0.0004	1.00	9	0.75	0.02	12	0.08	0.81	8	0.09	0.83
CBF % of CO	14	-0.20	0.49	14	0.22	0.45	13	0.20	0.52	9	0.17	0.65	12	-0.21	0.52	8	-0.55	0.16
CRP	8	0.06	0.90	8	-0.35	0.39	8	-0.38	0.36	3	-	-	6	-0.72	0.11	-	-	-
IL-6	9	0.08	0.85	9	0.29	0.45	9	0.62	0.07	4	-	-	8	-0.14	0.75	3	-	-
IL-8	9	-0.73	0.03	9	-0.42	0.27	9	0.62	0.09	4	-	-	8	0.02	0.98	3	-	-
IL-10	9	-0.33	0.39	9	0.57	0.12	9	0.57	0.12	4	-	-	8	0.05	0.93	3	-	-
IL-17	9	-0.15	0.71	9	0.73	0.02	9	0.25	0.52	4	-	-	8	0.32	0.44	3	-	-
MPO	9	-0.33	0.38	9	0.07	0.86	9	0.71	0.03	4	-	-	8	-0.27	0.52	3	-	-
TNF-a	9	-0.33	0.39	9	0.57	0.12	9	0.57	0.12	4	-	-	8	0.05	0.93	3	-	-

Baseline parameter	ssBRST overall			ssBRSA overall			scBRS overall			BEI overall			HVR			HVR int			HVR step		
	n	R	P	n	R	p	n	R	p	N	R	p	n	R	p	n	R	p	n	R	p
scBRS overall	6	0.36	0.48	6	0.25	0.63															
scBRS rising	6	0.26	0.62	6	0.30	0.57															
scBRS falling	3	-	-	3	-	-															
BEI overall	11	-0.28	0.40	11	-0.15	0.65															
BEI rising	11	0.08	0.82	11	-0.11	0.75															
BEI falling	11	0.08	0.81	11	-0.20	0.16															
pcBRS overall	5	-0.10	0.95	5	-0.10	0.95	3	-	-												
Transduction	10	-0.06	0.87	10	-0.05	0.89	6	-0.61	0.20	9	0.20	0.61									
HVR overall	9	-0.12	0.76	9	-0.09	0.82	6	-0.09	0.92	11	0.33	0.33									
HVR intermittent	7	-0.78	0.04	7	-0.45	0.31	3	-	-	6	0.62	0.19									
HVR stepped	2	-	-	2	-	-	3	-	-	5	-0.43	0.48									
Total CBF	11	-0.09	0.79	11	0.001	1.00	7	-0.22	0.64	13	0.26	0.38	10	-0.04	0.92	7	-0.07	0.87	3	-	-
CBF % of CO	11	-0.23	0.49	11	-0.13	0.70	7	0.15	0.75	13	0.30	0.32	10	-0.36	0.31	7	-0.22	0.64	3	-	-
CRP	4	-	-	4	-	-	4	-	-	7	-0.51	0.25	6	0.75	0.10	-	-	-	4	-	-
IL-6	6	-0.02	0.97	6	-0.16	0.77	4	-	-	9	-0.10	0.80	8	-0.13	0.76	4	-	-	4	-	-
IL-8	6	0.20	0.71	6	0.20	0.71	4	-	-	9	-0.02	0.98	8	-0.26	0.54	4	-	-	4	-	-
IL-10	6	-0.14	0.80	6	-0.14	0.80	4	-	-	9	-0.48	0.19	8	0.00	1.00	4	-	-	4	-	-
IL-17	6	-0.51	0.30	6	-0.64	0.17	4	-	-	9	0.04	0.92	8	-0.17	0.69	4	-	-	4	-	-
MPO	6	0.06	0.92	6	0.09	0.86	4	-	-	9	-0.45	0.23	8	-0.52	0.19	4	-	-	4	-	-
TNF-a	6	-0.14	0.80	6	-0.14	0.80	4	-	-	9	-0.48	0.19	8	0.00	1.00	4	-	-	4	-	-

8.4 Appendix 4. Univariate binomial logistic regression data

Binomial logistic regression performed using the BP response to RDN as the dependent variable (response defined as ≥10 mmHg reduction in office systolic blood pressure at 6 months post-RDN). oSBP; office systolic blood pressure, oPP; office pulse pressure, eGFR; estimated glomerular filtration rate, iLVM; left ventricular mass indexed to body surface area, MSNA; muscle sympathetic nerve activity, ssBRSA; spontaneous sympathetic vascular baroreflex sensitivity quantified using the area method, scBRS; spontaneous cardiac baroreflex sensitivity. The Nagelkerke R^2 quantifies the variance attributed to each variable. The correct classification describes the percentage of patients correctly classified as responders or non-responders by the model. The B value indicates the size of the effect of an independent variable on the dependent variable (RDN response), and for categorical data would represent the odds ratio, the 95% confidence interval is shown in parentheses.

Baseline variable		Chi-squared		Nagelkerke	Correct classification	Prediction of effect/odds ratio	
	N	X^2	p	R^2	%	Exp(B)	p
Age	18	0.781	0.377	0.058	72	0.960 (0.876 – 1.053)	0.387
Gender	18	0.234	0.628	0.018	61	0.625 (0.093 – 4.222)	0.630
oSBP	18	11.688	0.001	0.648	83	1.148 (1.012 – 1.303)	0.031
oPP	18	1.206	0.272	0.088	67	1.029 (0.975 – 1.087)	0.298
eGFR	18	0.139	0.709	0.010	61	1.017 (0.931 – 1.111)	0.710
iLVM	18	0.122	0.727	0.009	61	1.007 (0.966 – 1.050)	0.730
Aortic distensibility	15	1.227	0.268	0.106	67	1.864 (0.568 – 6.120)	0.304
MSNA	14	1.239	0.266	0.114	64	0.969 (0.915 – 1.027)	0.288
ssBRSA	13	2.421	0.120	0.231	62	0.111 (0.005 – 2.663)	0.175
scBRS	13	2.119	0.145	0.212	69	0.832 (0.638 – 1.085)	0.175
Transduction	13	0.475	0.491	0.049	69	0.034 (0.000 – 684.475)	0.503

8.5 Appendix 5. Drugs present on retrospective analysis of urinary anthypertensive drug metabolites

Stored urine sampes were sent for analysis of antihypertensive drug metabolites. The metabolities screened for were: Amlodipine, Diltiazem, Felodipine, Lisinopril, Perindopril, Ramipril, Losartan, Irbesartan, Candesartan, Indapamide, Furosemide, Bendroflumethiazide, Hydrochlorothiazide, Atenolol, Labetolol, Bisoprolol, Doxazosin, Enalapril, Metoprolol, Nifidepine, Verapamil, Spironolactone metabolite and Moxonidine. Aliskiren, Carvedilol and Tadalafil were not screened for. On previous analyses Nifedipine and Felodipine appear to be unstable, and therefore the analyses below do not take into account the prescription of these latter agents.

There were no significant changes in the average number of medications prescribed (Px) or detected in the urine over the course of the study (ANOVA p=0.72 and p=0.59, respectively). There was no change in the percentage of prescribed medications that were detected over the course of the study (ANOVA p=0.41). Data shown as mean ± SEM (standard error of the mean).

Patient No.	Time post RDN (months)											
	0			1			6			12		
	Px	Detected	% Detected	Px	Detected	% Detected	Px	Detected	% Detected	Px	Detected	% Detected
4	4	0	0	2	2	100	3	0	0	1	0	0
6	4	0	0	4	0	0	4	0	0	4	3	75
12	3	3	100	3	3	100	3	2	67	3	3	100
13	6	4	67	5	5	100	5	3	50	5	5	100
14	3	3	100	3	3	100	2	2	100	2	2	100
18	3	3	100	3	3	100	3	3	100	3	3	100
Mean	3.8	2.2	61.1	3.3	2.7	83.3	3.3	1.7	54.4	3.0	2.7	79.2
SEM	0.5	0.7	20.0	0.4	0.7	16.7	0.4	0.6	18.5	0.6	0.7	16.4